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Stable potassium isotope distribution in mouse organs and red blood cells: implication for biomarker development

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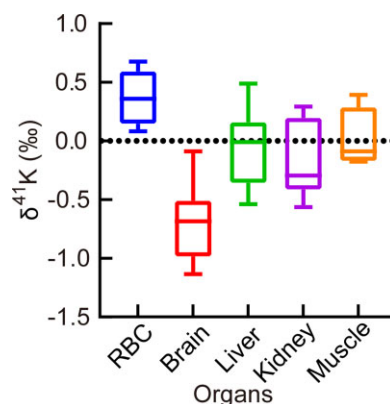
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Abstract

Potassium (K) is an essential electrolyte for cellular functions in living organisms, and disturbances in K⁺ homeostasis could lead to various chronic diseases (e.g. hypertension, cardiac disease, diabetes, and bone health). However, little is known about the natural distribution of stable K isotopes in mammals and their application to investigate bodily homeostasis and/or as biomarkers for diseases. Here, we measured K isotopic compositions ($\delta^{41}\text{K}$, per mil deviation of $^{41}\text{K}/^{39}\text{K}$ from the NIST SRM 3141a standard) of brain, liver, kidney, and red blood cells (RBCs) from 10 mice (five females and five males) with three different genetic backgrounds. Our results reveal that different organs and RBCs have distinct K isotopic signatures. Specifically, the RBCs have heavy K isotopes enrichment with $\delta^{41}\text{K}$ ranging from 0.67 to 0.08‰, while the brains show lighter K isotopic compositions with $\delta^{41}\text{K}$ ranging from -1.13 to -0.09 ‰ compared to the livers ($\delta^{41}\text{K} = -0.12 \pm 0.58$ ‰) and kidneys ($\delta^{41}\text{K} = -0.24 \pm 0.57$ ‰). We found that the K isotopic and concentration variability is mostly controlled by the organs, with a minor effect of the genetic background and sex. Our study suggests that the K isotopic composition could be used as a biomarker for changes in K⁺ homeostasis and related diseases such as hypertension, cardiovascular, and neurodegenerative diseases.

Keywords: K isotopes, K concentration, mouse organs, sex, genetic background

Graphical abstract



K isotopic composition of red blood cell (RBC), brain, liver, kidney and muscle of mice.

Introduction

Metal ions play an important role in synthesizing biomolecules and maintaining their proper functions.¹ Biochemical processes may induce isotopic fractionations that lead to different isotopic compositions of a given metal between body compartments. For metal ions, these isotopic effects are often limited to sub-per mil

levels, thus requiring advanced mass-spectrometry techniques to sufficiently resolve such differences. The advent of high-precision multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) offers the precision required to resolve such isotopic fractionations,^{2,3} and metal stable isotopes have been increasingly studied in biochemistry and medical science.⁴

Stable isotope studies of transition metals (e.g. Fe, Cu, and Zn) and alkaline earth metals (Mg and Ca) show great promise to be used as clinic diagnostic tools for diseases related to disorders in metal homeostasis.^{5–8} For instance, Cu isotopes have been used to track liver cirrhosis,^{9–11} cancers,^{12,13} and neurodegenerative diseases^{8,14,15}; zinc isotopes are a potential indicator of cancers^{16–19}; iron isotopes are considered a tracker of hereditary diseases²⁰; calcium isotopes have been used as a diagnostic tool for bone diseases^{21–23} and potentially for neurodegenerative diseases.⁸ Furthermore, the natural distribution of Cu, Zn, Fe, and Ca isotopes in organs and body fluids from different mammals has been reported, and modified distributions could be used as tracers of various diseases.^{6–8,15,24}

Potassium (K) is the most abundant intracellular cation in living organisms. Within the body, ~98% of K is in the intracellular fluid compartments.^{25,26} It regulates the appropriate osmotic pressure and acid-base balance between body fluids and tissues, and participates in the metabolism of sugars and proteins in cells. Therefore, K plays a critical role in transmitting nerve impulses, cardiac activity, membrane transport, and neuromuscular functions.^{25,27} Scientific and clinical studies have shown that a K-rich diet can help patients to achieve lower blood pressure and slow the progression of kidney diseases.^{28,29} Increased K intake has also been suggested to be linked to a reduced risk of neurodegenerative diseases such as Alzheimer's disease (AD) and related dementia.³⁰ Major pathological features of AD include the formation of neurofibrillary tangles by neuronal accumulations of abnormally hyperphosphorylated tau filaments, and the formation of senile plaques by extracellular deposits of amyloid β (A β) fibrils.^{31,32} Preliminary studies of the isotopic compositions of Cu, Fe, and Zn associated with AD in body compartments have shown the potential of these isotope systematics in neurodegenerative diseases.^{8,15,33} In contrast with the accumulation of Cu, Fe, and Zn in AD patients' brains, the concentration of K shows a significant decrease in the brain with a concomitant increase in serum, indicating signal transferal from the brain to blood.³⁴ Exploration of K isotopic variations in body compartments is particularly important for its application in biomedicine.

Potassium has two stable isotopes (³⁹K, 93.258% and ⁴¹K, 6.730%) with a relatively large mass difference (5%),³⁵ indicating that potentially large mass-dependent fractionation may occur in a variety of geochemical and biochemical processes. Analysis of K isotopes has been plagued by intensive Ar-related interferences. Nevertheless, there is a renewed interest in K isotope geochemistry driven by the development of high-precision analytical methods using MC-ICP-MS, and it has been widely explored in the fields of geochemistry and cosmochemistry.³⁶ Studies of K stable isotopes in biological systems, however, remain limited and are mainly focused on terrestrial plants.^{37,38}

The Nu Sapphire MC-ICP-MS utilizes an improved collision cell system to minimize the influence of Ar hydrides,^{39–42} and has been used successfully to analyze the K isotopic compositions of biological materials.⁴³ In that work,⁴³ reported a large K isotopic variation for seven representative biological reference materials (land and sea animals and bodily organs), suggesting that K isotopes are useful for studying biochemical metal-related processes. Additionally,⁴⁴ used MC-ICP-MS in extra-high resolution mode to demonstrate variations in K isotopic compositions among land and sea creatures and human-derived biofluid reference materials. High K concentrations in most bodily reservoirs make experiments easier to achieve than transition metals (e.g. Fe, Cu, and Zn), and additionally ease issues with contamina-

tion that can be a significant consideration for the metals listed above.⁴⁵ The potential distinct K isotopic composition of individual organs could be used to detect abnormal metabolism of K caused by diseases. Consequently, it is necessary to evaluate the effect of K concentration, genetic background, and sex on K isotopic composition in the bodily reservoirs.

Using the Sapphire MC-ICPMS, we report the first systematic study of K isotopic distribution in organs (brain, liver, kidney, and muscle) and blood [red blood cells(RBCs)] from 10 mice. We examine the effects of organs, genetic backgrounds, and sex on K isotopes in mice. Our results provide a baseline for K-stable isotopic compositions in the healthy body, which is critical to future studies of diseases and the development of biomarkers.

Experimental Sample collection

Three-month-old mice studied here comprise five females and five males. They were raised under specific pathogen-free conditions at LVTS INSERM 1148, Hospital Bichat, Paris, and are the same materials for which Cu isotopic compositions have been measured previously.¹⁵ The 10 mice were derived from three different genotypes, including C57BL/6JrJ, BALB/cJrJ, and 129S2/SvPasOrlJ. They were kept on the same diet based on dry food from birth. All experiments on mice were conducted according to the Directive 2010/63/EU of the European Parliament, and this study has obtained review and approval from the Comité d'Ethique Paris Nord #121 (APAFIS#15229). The mice were sacrificed by exsanguination using an overdose of ketamine and xylazine, and the organs were collected and processed as described in Reference¹⁵.

Sample digestion and chemical purification

Sample digestion and chemical purification were carried out in a clean laboratory at the Institut de Physique du Globe de Paris (IPGP). Chemical procedures were performed in class 100 vented laminar flow hoods in a class 10 000 clean laboratory. Milli-Q water, Saville screw-top polyfluoroalkyl (PFA) beakers, and other materials were cleaned to minimize the blank level. Between 10 and 20 mg of organs and blood were digested using a mixture of concentrated HNO₃ and Seastar optima-grade H₂O₂ in closed PFA beakers to remove organics. Samples were then heated at 100°C for one to two days until complete digestion. About 20 mg of basalt standard BHVO-2 from the United States Geological Survey (USGS) were dissolved in a mixture of concentrated HF-HCl-HNO₃ and heated on a hotplate until complete dissolution.

The K column chemistry was achieved by cation exchange chromatography, following the procedure of Reference⁴⁶. A total of 10% of each biological sample was taken and dissolved in 1 ml of 0.5 N HNO₃. All columns were packed with 2 ml of pre-cleaned Bio-Rad AG 50W-X8 cation exchange resin (200–400 mesh) and conditioned by 10 ml of 0.5 N HNO₃. Sample solutions were then loaded on the resin, and matrix elements were eluted by passing 13 ml of 0.5 N HNO₃. The K fraction was subsequently collected in 22 ml of 0.5 N HNO₃, and the remaining elements in the resin were washed off with 10 ml of 6 N HCl. The same separation procedure was repeated twice to ensure complete matrix removal. Finally, the collected K fraction was dried down at 120°C in preparation for isotope measurements.

Potassium isotope measurements on the Sapphire CC-MC-ICP-MS

The K isotopic compositions were measured on the Sapphire CC-MC-ICP-MS instrument. Analytical details have been reported in Reference ³⁹ and applied in Reference ⁴³ in biological materials, and remain the same here. Instrument settings were tuned to optimize the stability of the instrumental mass bias and K intensity. The flow rates of H₂ (reaction gas) and He (buffer gas) introduced into the collision cell are optimized to minimize hydride interferences. A blank solution of 3% HNO₃ was measured at the beginning of each analytical session and then subtracted from the measured intensities of ion beams.

Samples and K standard (NIST SRM3141a) were dissolved using the same acid (3% HNO₃) and matched to better than 1%. The solutions were introduced into the mass spectrometer using an Apex Omega desolvating system fitted with a 100 μl/min PFA nebulizer (MicroFlow nebulizer, Elemental Scientific, Omaha, NE, USA) and with standard Ni dry cones. Isotope ratios were measured using the conventional standard-sample bracketing (SSB) method, which consists of measuring a standard before and after each sample and using the average of the two standards for the normalizing ratio. The K isotope compositions are expressed in delta (δ) notation relative to the bracketing K standard, which is defined as follows:

$$\delta^{41}\text{K} (\text{‰}) = \left[\frac{(^{41}\text{K}/^{39}\text{K})_{\text{sample}}}{(^{41}\text{K}/^{39}\text{K})_{\text{standard}}} - 1 \right] \times 1000.$$

The reported $\delta^{41}\text{K}$ value is the average of N repeated analyses for each sample, and analytical uncertainty is calculated as 2SD (standard deviation) of the repeated analyses. The biological standard (ERM-BB186, pig kidney) and basalt standard (BHVO-2) were analyzed during each analytical session as quality control, which have been analyzed on this instrument multiple times.^{39,43}

Statistical analysis

Statistical analysis was conducted using GraphPad Prism, where two-way ANOVAs were used to evaluate the effects of various factors (sex, genotype, and organs), followed by Sidak's multiple comparison test for paired data sets. Multiple linear regression was performed to test the impact of various factors upon $\delta^{41}\text{K}$. Statistical significance is defined by P-values <0.05 (denoted by an asterisk, *), with more asterisks corresponding to ever-higher levels of significance (**P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). Data are presented as box-and-whiskers graphs, with the box extending from the 25th to 75th percentiles, the line representing the median, and the whiskers showing the minimal and maximal values. Furthermore, the Spearman and Pearson correlation was used to analyze non-normally distributed data and normally distributed data, respectively. The Shapiro–Wilk test was used to determine the normality of data and whether it is appropriate to use normal distribution-based statistical methods, such as ANOVA.

Results

The K isotopic compositions and elemental concentrations of different organs (brain, liver, kidney, and muscle) and RBCs are shown in Table 1. The average $\delta^{41}\text{K}$ values of ERM-BB186 (−0.32 ± 0.09‰) and BHVO-2 (−0.40 ± 0.07‰) from different analytical sessions are consistent with literature data^{39,43,46} (e.g. Xu et al., 2019; Moynier et al., 2021a,b), indicating our K isotopic data are accurate. A multifactorial variance analyses of the $\delta^{41}\text{K}$ (Table 2) shows that 64.7% of the variability is carried by the organs (P < 0.0001), with minor contributions from the sex

(4.2%, P = 0.0183) and the strain (4.5%, P = 0.0465) and none for the K concentration (0.7%, P = 0.3153). For the K concentrations, multifactorial variance analyses (Table 3), shows that only the strain has a significant effect (21% of the variability, P = 0.007).

K isotopic distribution in different compartments of mice

Large variation of K isotopic compositions was found in the body compartment of mice, which spans almost 1.81‰ from the RBC of mouse 6 (0.67 ± 0.12‰) to the brain of mouse 10 (−1.13 ± 0.07‰) (Table 1). RBCs of different mice show variable but consistently heavy isotopic compositions ($\delta^{41}\text{K}$ = 0.08–0.67‰) relative to brain samples ($\delta^{41}\text{K}$ = −1.13 to −0.09‰). Liver, kidney, and muscle have slightly different average $\delta^{41}\text{K}$ values of −0.12 ± 0.58‰, −0.24 ± 0.57‰, and 0.01 ± 0.53‰, respectively (Fig. 1). The correlations between $\delta^{41}\text{K}$ values of different organs and RBCs were analyzed using a Spearman correlation matrix (Fig. 2). A higher correlation is observed between K isotopic compositions in brain and RBC, as well as liver and kidney (Spearman's coefficients of 0.95 and 0.83, respectively) than in other organs (Fig. 2). The strong linear correlation of $\delta^{41}\text{K}$ values between brain and RBC was shown with an R² value of 0.62 (Pearson's correlation coefficient, Fig. 3A). A similar positive linear correlation was seen for $\delta^{41}\text{K}$ values between liver and kidney (R² = 0.39) (Fig. 3B). Moreover, the correlations of $\delta^{41}\text{K}$ in the two pairs reached statistical significance with P-values < 0.05 (Fig. 3A and B), whereas $\delta^{41}\text{K}$ values in other pairs of organs did not display a statistical significance.

K isotopic compositions in mice of different genetic background

The K isotopic compositions of the mice show apparent variations in different genetic backgrounds (C57BL/6JRj, BALB/cJRj, and 129S2/SvPasOrlRj) (Fig. 4). There was not enough data to analyze the statistical significance between the muscle samples of each strain (Table 1). The genetic background accounts for 9.94% of overall variation, which is much lower than the difference due to the organ nature (61.03% of total variation). The brain and liver of strain C57BL/6JRj mice have higher $\delta^{41}\text{K}$ values than those with other genetic backgrounds (Fig. 4). The $\delta^{41}\text{K}$ values of different organs of strain BALB/cJRj mice partially overlap with those of strain C57BL/6JRj mice (Fig. 4). The range of most $\delta^{41}\text{K}$ values of different organs on 129S2/SvPasOrlRj mice overlaps with those on BALB/cJRj mice (Fig. 4). Overall, the $\delta^{41}\text{K}$ values of each organ did not show statistically significances between the three genetic backgrounds (Fig. 4).

K isotopic compositions in female and male mice

Organs and RBCs in female mice are apparently enriched in heavy K isotopes relative to male ones (Fig. 5). The sex accounts for 10.4% of all variations, which is much lower than the variations between organs (65.8%). The most striking variations are observed in brains between female ($\delta^{41}\text{K}$ = −0.09 to −0.7‰) and male ($\delta^{41}\text{K}$ = −0.63 to −1.13‰) (P-value of 0.015). RBC and liver in females show slightly higher $\delta^{41}\text{K}$ values than those in males (P-value > 0.05; Fig. 5). It is attributed to the mouse 18 which has higher $\delta^{41}\text{K}$ values ($\delta^{41}\text{K}$ = 0.59 ± 0.15‰) than those in other males ($\delta^{41}\text{K}$ = 0.08–0.30‰) (Fig. 6; Table 1). The origin of individual differences in RBC of mouse 18 is unknown, as its other organs show similar $\delta^{41}\text{K}$ values to other males. The distinct $\delta^{41}\text{K}$ values of kidneys between females and males are shown, but this is not statistically significant (P-value of 0.057). It is mainly controlled by the difference

Table 1. Potassium isotopic compositions of different organs and RBC in mice

Mouse number	Organs	$\delta^{41}\text{K}$ (‰)	2SD (‰)	N	K (ppm)	Strain	Sex
4	RBC	0.61	0.13	4	7751	C57BL/6Jrj	F
6	RBC	0.67	0.13	4	5562	BALB/cJrj	F
22	RBC	0.51	0.08	4	5899	C57BL/6Jrj	F
28	RBC	0.42	0.04	4	3506	BALB/cJrj	F
30	RBC	0.14	0.03	4	2569	129S2/SvPasOrlRj	F
Average		0.47					
2	RBC	0.18	0.07	6	7365	129S2/SvPasOrlRj	M
3	RBC	0.08	0.06	4	3638	BALB/cJrj	M
10	RBC	0.30	0.08	4	3420	BALB/cJrj	M
11	RBC	0.15	0.06	5	10 943	129S2/SvPasOrlRj	M
18	RBC	0.59	0.15	4	2758	C57BL/6Jrj	M
Average		0.26					
4	Brain	-0.09	0.03	4	6422	C57BL/6Jrj	F
6	Brain	-0.34	0.02	4	4490	BALB/cJrj	F
22	Brain	-0.57	0.04	4	6335	C57BL/6Jrj	F
28	Brain	-0.67	0.07	4	5902	BALB/cJrj	F
30	Brain	-0.70	0.07	4	6123	129S2/SvPasOrlRj	F
Average		-0.47					
2	Brain	-0.94	0.09	5	12 906	129S2/SvPasOrlRj	M
3	Brain	-0.97	0.06	7	6629	BALB/cJrj	M
10	Brain	-1.13	0.07	3	3370	BALB/cJrj	M
11	Brain	-0.96	0.08	6	9709	129S2/SvPasOrlRj	M
18	Brain	-0.63	0.10	4	8764	C57BL/6Jrj	M
Average		-0.93					
4	Liver	0.42	0.12	6	3506	C57BL/6Jrj	F
6	Liver	0.10	0.13	4	4310	BALB/cJrj	F
22	Liver	-0.16	0.09	4	4510	C57BL/6Jrj	F
28	Liver	-0.05	0.07	4	5843	BALB/cJrj	F
30	Liver	-0.54	0.05	4	3522	129S2/SvPasOrlRj	F
Average		-0.05					
2	Liver	0.03	0.08	5	11 215	129S2/SvPasOrlRj	M
3	Liver	-0.50	0.07	4	3797	BALB/cJrj	M
10	Liver	-0.31	0.07	4	4216	BALB/cJrj	M
11	Liver	-0.22	0.09	5	7516	129S2/SvPasOrlRj	M
18	Liver	0.07	0.16	4	4115	C57BL/6Jrj	M
Average		-0.19					
4	Kidney	0.18	0.11	4	3641	C57BL/6Jrj	F
6	Kidney	0.27	0.10	8	4367	BALB/cJrj	F
22	Kidney	-0.29	0.18	4	3509	C57BL/6Jrj	F
30	Kidney	-0.39	0.03	4	5962	129S2/SvPasOrlRj	F
Average		-0.06					
2	Kidney	-0.50	0.13	6	7670	129S2/SvPasOrlRj	M
3	Kidney	-0.30	0.15	4	9626	BALB/cJrj	M
10	Kidney	-0.56	0.09	4	2663	BALB/cJrj	M
11	Kidney	-0.29	0.04	5	10 060	129S2/SvPasOrlRj	M
18	Kidney	-0.31	0.22	4	2568	C57BL/6Jrj	M
Average		-0.39					
4	Muscle	-0.18	0.03	4	4440	C57BL/6Jrj	F
6	Muscle	-0.15	0.09	4	8540	BALB/cJrj	F
Average		-0.16					
11	Muscle	0.39	0.08	4	5352	129S2/SvPasOrlRj	M
18	Muscle	-0.03	0.18	4	5636	C57BL/6Jrj	M
Average		0.18					

of mouse 4 and 6 with high $\delta^{41}\text{K}$ values in all organs of the female (Fig. 6). It is important to highlight that a recent study conducted by⁴⁷ has identified isotopic heterogeneity of K within different brain regions, which could potentially explain some of the variability observed in this study.

K elemental concentration

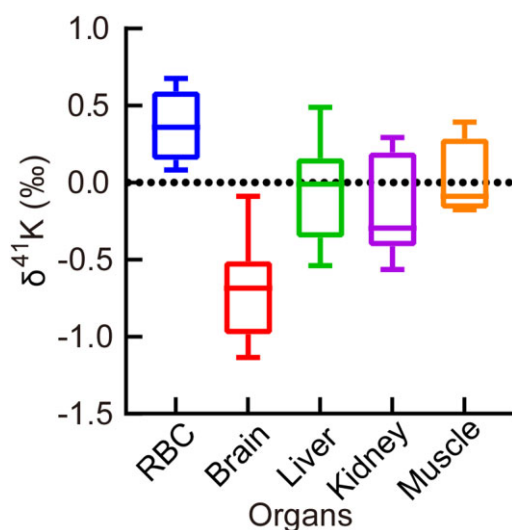
Potassium concentrations in this study have been estimated on the CC-MC-ICPMS by comparing sample K voltage to that of the standard NIST SRM3141a of a known concentration (Table 1). The K concentrations vary among different organs, with the

Table 2. Multiple linear regression (least square methods) for $\delta^{41}\text{K}$

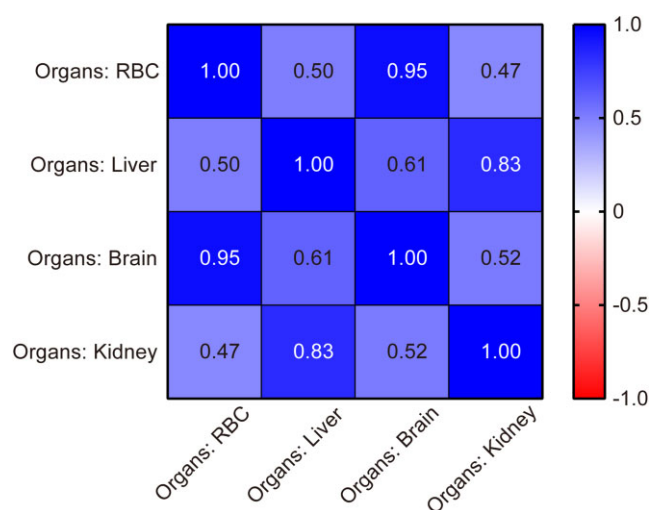
Analysis of variance	SS	P-value	% variation
Regression	6.808	<0.0001	77.0
Organs	5.717	<0.0001	64.7
Sex	0.3674	0.0183	4.2
Strain	0.402	0.0465	4.5
[K] (ppm)	0.0621	0.3153	0.7
Residual	2.033		
Total	8.84		

Table 3. Multiple linear regression (least square methods) for K concentration

Analysis of variance	SS	P-value	% variation
Regression	106 314 388	0.0288	37.2
Strain	60 913 356	0.0070	21.3
Sex	12 963 512	0.1264	4.5
$\delta^{41}\text{K}$ sample (‰)	5483 632	0.3153	1.9
Organs	24 144 781	0.3528	8.4
Residual	179 475 410		
Total	285 789 798		

**Fig. 1** K isotopic composition of RBC, brain, liver, kidney, and muscle of mice.

brain showing higher levels compared to other organs (Fig. 7). There were no systematic variations in K concentrations between female and male organs, and the differences observed were not statistically significant (Fig. 8). Notably, the K concentration in RBC was higher in female mice than in male mice, while the concentration in the livers of female mice was slightly higher than that in males. In contrast, the K concentrations in the brains and kidneys of female mice were significantly lower than those of male mice (Fig. 8). Furthermore, genetic background has no any significant effect on the K concentration (Fig. 9).

**Fig. 2** Spearman correlation matrix for K isotopic compositions of different organs and RBC.

Discussion

Here we focus on the effects of different organs, genetic background, sex, and K concentration on the K isotopic variations in mice.

K isotopic difference and effect in different organs and RBC

Equilibrium isotope fractionation occurs due to vibrational energy differences between the bonds formed by the isotopes of a given element, and heavy isotopes are preferentially inhabited at stronger bonding sites to minimize system energy.^{48,49} The lighter isotopes of an element form weaker bonds and diffuse faster during kinetic fractionation.⁵⁰ The mouse organs have distinct K isotopic compositions with RBC enriched in heavy isotopes, and brain, liver, and kidney enriched in light isotopes (Figs. 1, 4, and 5). Their K isotopic variations can be explained by the change in the K bonding environment,⁵¹ led to conclude that K from different organs of humans and animals is much more active than ordinary mineral K. On the cellular level, the Na/K-ATPase is an essential ion pump that maintains membrane potentials and Na and K concentration gradients in all mammalian cells.^{52,53} The Na/K-ATPase drives the extrusion of three Na⁺ ions from the cell and the uptake of two K⁺ ions by hydrolyzing one adenosine triphosphate (ATP) molecule.^{54,55} The general rule that heavy isotopes preferentially combine with stronger bonds would predict that K isotopes bound to Na/K-ATPase should be isotopically heavier than extracellular free/hydrated K⁺. The K⁺ ions in RBC are mostly bounded to ATPase proteins to form Na/K-ATPase and thus should be enriched in heavy K isotopes, which is consistent with the K isotopic composition signature in this study (RBC: $\delta^{41}\text{K} = 0.08\text{--}0.67\text{‰}$; Fig. 1).

Oxidative metabolism is very active in the brain, where large amounts of chemical energy as ATP molecules are consumed to keep the balance of nerve impulse propagation, neurotransmitter release, and cation homeostasis.⁵⁵ At the nervous system level, protein phosphorylation is the major molecular mechanism through which the function of neural proteins is modulated in response to extracellular signals, such as the response to neurotransmitter stimuli. The binding of Na⁺ on the cytoplasmic site triggers phosphorylation of the enzymes from ATP, in contrast to the extracellular binding of K⁺, which triggers rapid

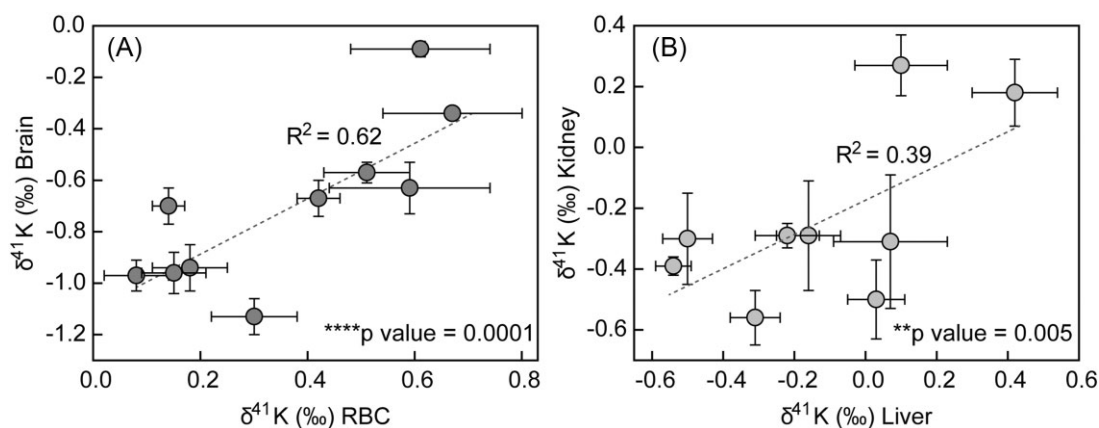


Fig. 3 Correlations of K isotopic compositions in brain versus RBC (A) and liver versus kidney (B). Coefficient of determination (R^2) and statistical significance of linear regression (P-values) between each pair are given in the figure.

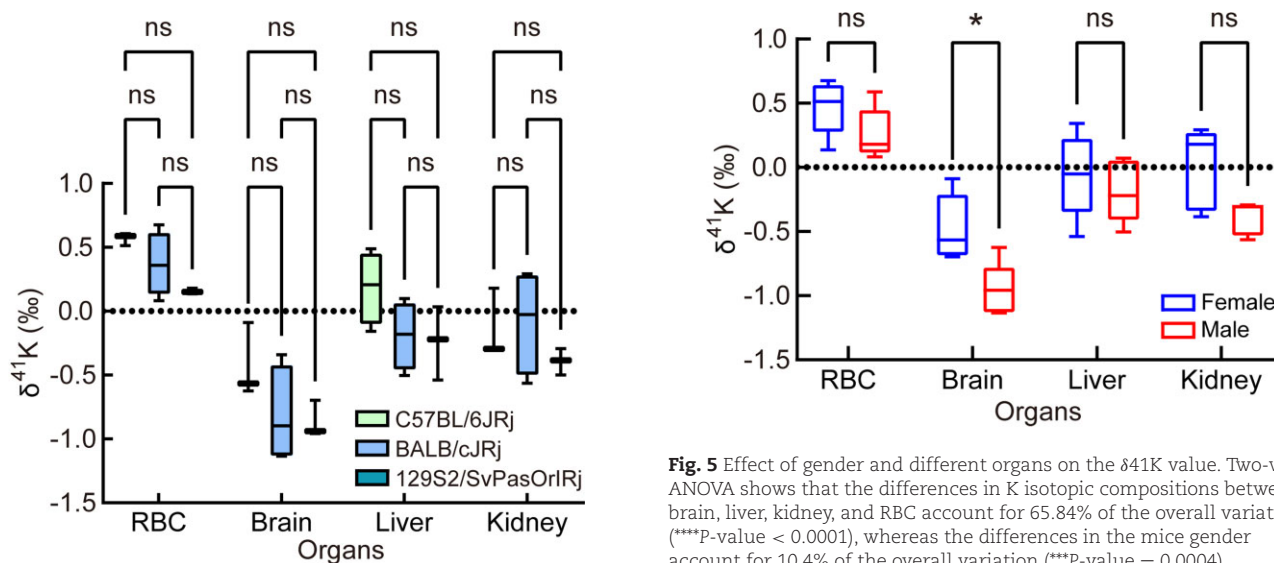


Fig. 4 Effect of the three genetic backgrounds and different organs on the $\delta^{41}\text{K}$ value. Two-way ANOVA shows that the differences in K isotopic compositions between brain, liver, kidney, and RBC account for 61.03% of the overall variation ($****P$ -value < 0.0001), whereas the differences in the three genetic backgrounds (C57BL/6JRj, BALB/cJRj, and 129S2/SvPasOrlRj) account for 9.94% of the overall variation ($**P$ -value = 0.0058).

dephosphorylation.⁵⁶ The phosphorylation of the Na/K-ATPase catalytic subunit inhibits enzyme activity, whereas the inhibition of protein kinase C restores the enzyme activity.⁵⁷ For the enrichment of the lighter K isotopes in the brain (Fig. 1), the change in Na/K-ATPase activity due to the loss of intracellular K may result in a decrease in brain $\delta^{41}\text{K}$ values with the loss of the heavy-isotope enriched Na/K-ATPase. Meanwhile, the K efflux into the bloodstream has a corresponding and mass-balance-modulated increase in the $\delta^{41}\text{K}$ value. It is consistent with the strong Spearman correlation of K isotopes between RBC and brain (Figs. 2 and 3), suggesting that the enrichment of light K isotopes in the brain directly reflects the enrichment of heavy isotopes in RBC.

Liver and kidney organs have similar average K isotopic compositions (liver: $\delta^{41}\text{K} = -0.07 \pm 0.31\%$; kidney: $\delta^{41}\text{K} = -0.19 \pm 0.56\%$; Fig. 1), and show a strong Spearman correlation (Fig. 2). This may suggest a similar K-bonding environment in them. The liver and kidney are important contributors to the control of the K balance

Fig. 5 Effect of gender and different organs on the $\delta^{41}\text{K}$ value. Two-way ANOVA shows that the differences in K isotopic compositions between brain, liver, kidney, and RBC account for 65.84% of the overall variation ($****P$ -value < 0.0001), whereas the differences in the mice gender account for 10.4% of the overall variation ($***P$ -value = 0.0004).

in the body. When high K intake in the body induced the fluctuation in plasma K concentration, the liver would buffer the changes in plasma K concentration using transcellular K redistribution, and further drive feedback control of kidney K excretion.⁵⁸ Moreover, the collecting duct cell type of the kidney mediates acid-base transport but upregulates expression of luminal H/K-ATPase during K depletion to enhance K reabsorption, thereby limiting K wasting.⁵⁹ Thus, the liver and kidney act as mediators in the body to restore the K balance through K excretion and reabsorption. Their buffer mechanisms downstream of K in animals or humans might explain the intermediate K isotopic composition compared to that in the brain and RBC. More meaningful is that the current work would provide the impetus for continued exploration of K isotopes in different brain regions and further clarify the application of K isotopes to neurological diseases.

K isotopic variation is not directly dependent on K concentration

Clinically, the serum K concentration has been used as an indicator to evaluate the K level in the body, and further to track some diseases induced by abnormal K concentration.⁶⁰ However, most of K is stored in cells with a small proportion of serum K. The serum K concentrations might be affected by many factors, such

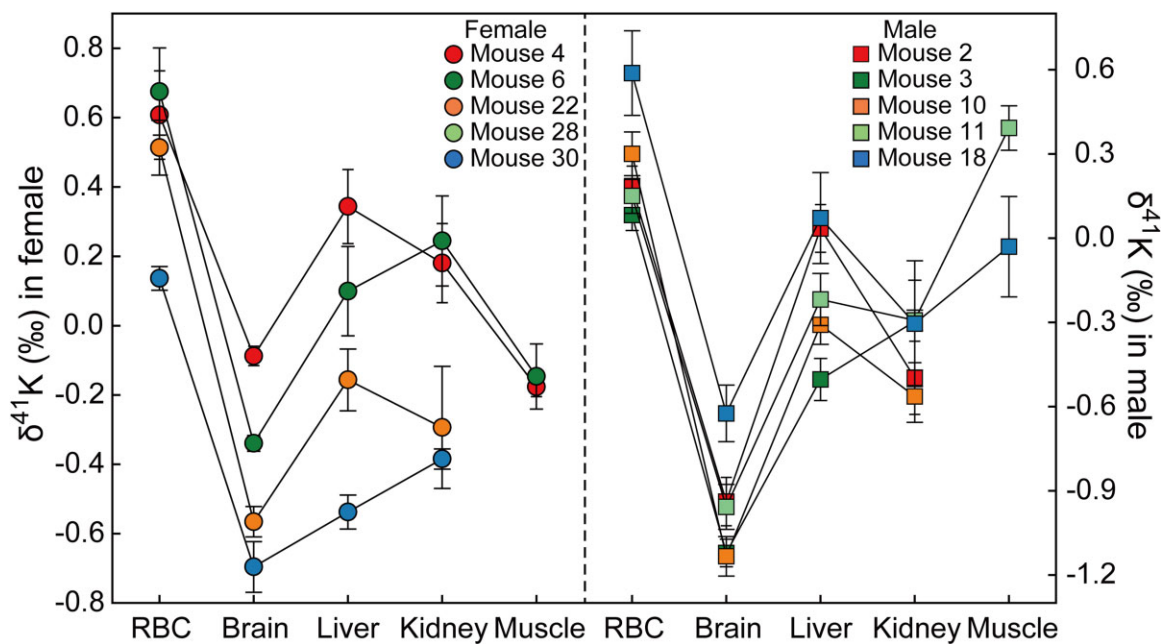


Fig. 6 K isotopic composition of organs and RBC in female and male mouse groups.

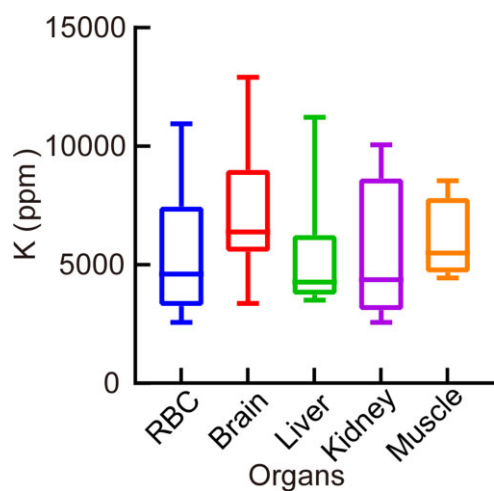


Fig. 7 K concentration in RBC, brain, liver, and kidney of mice.

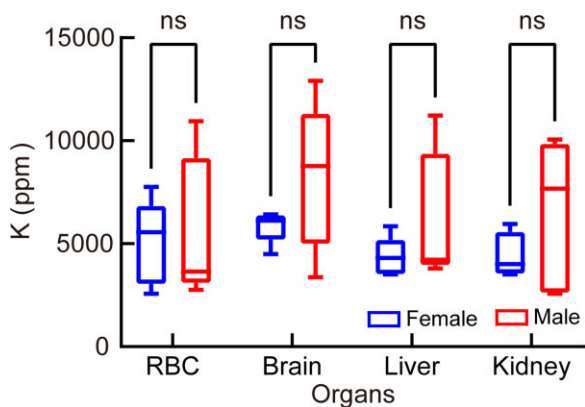


Fig. 8 K concentration in different organs and RBC from female and male mice. Two-way ANOVA shows that there is no significant difference between males and females.

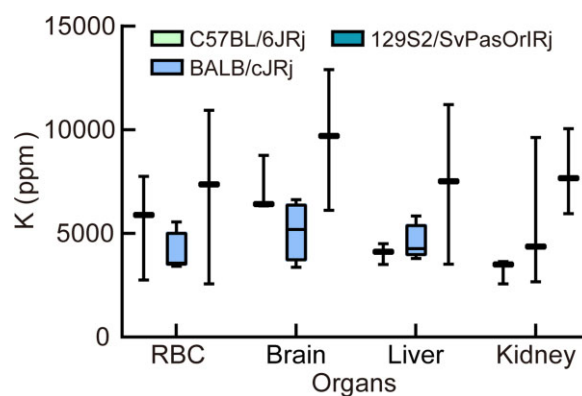


Fig. 9 The effect of the three genetic backgrounds and different organs on the K concentration. Two-way ANOVA shows that there is no significant difference between the three genetic backgrounds.

as dietary intake of K, absorption and consumption of K within the body, and the external environment.⁶¹ As shown in the result section, there is no K concentration control on the K isotopic variations. This is also illustrated by the fact that the different organs analyzed here do not show any statistical difference in their K concentration (Fig. 7). Given that K concentration in serum is much lower than in the RBC,⁶² it is more susceptible to concentration and isotopic excursions from brain influx. A strong correlation between brain and RBC (Figs. 2 and 3) suggests that the above effect could be exacerbated in serum. Both RBC and serum should be further investigated for K isotope systematics and their possible utility in diagnostics.

The K concentration is different in the male organs compared to the female ones (Fig. 8), and this difference is likely related to K metabolism between sexes due to these mice's growth in a controlled environment. The K concentration in male brains is significantly higher than in female brains (Fig. 7). A previous study found that K levels were significantly decreased across all intracellular compartments within AD patients' brains,³⁴ and females

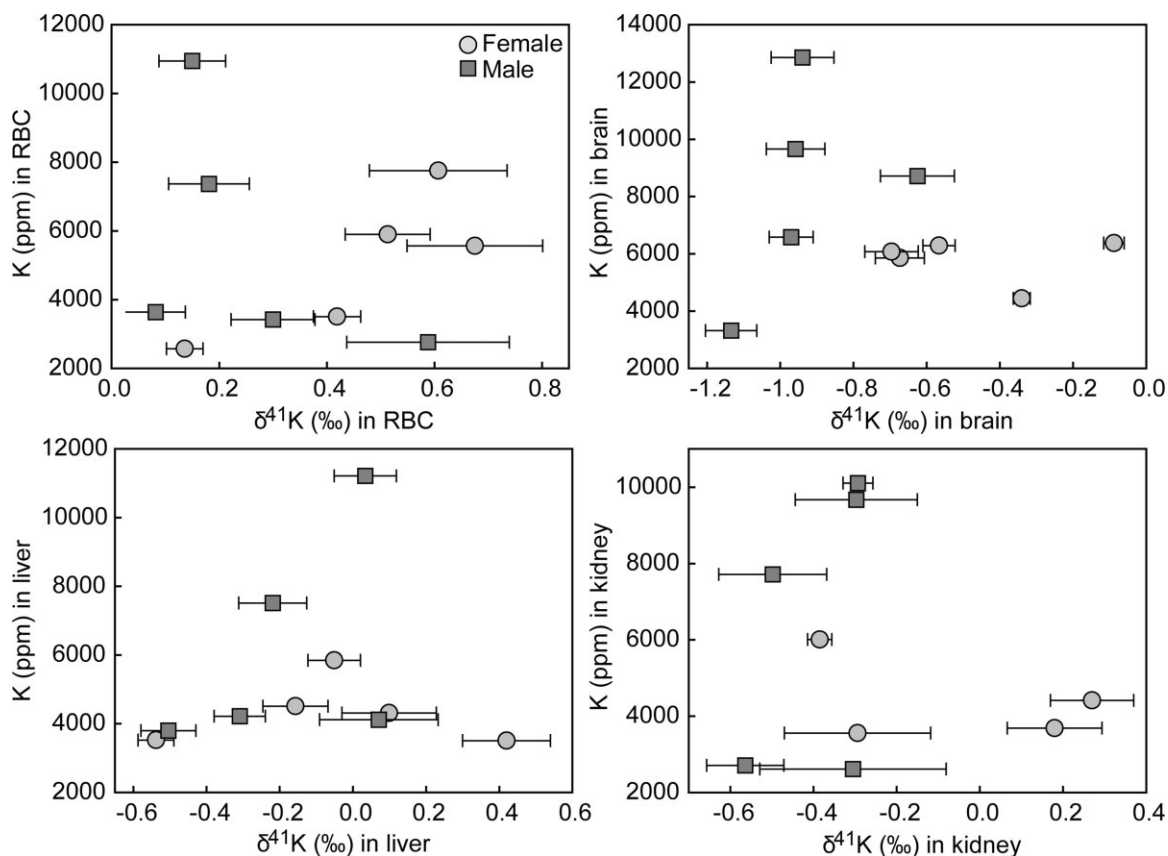


Fig. 10 Correlations between K isotopic composition and K concentration of different organs and RBC in mice.

are more prone to develop AD than males.³² Such concentration changes could be accompanied by an isotopic imbalance, with a resultant fractionation of K isotopes between the brain and other organs. A significant decrease in brain K concentration with AD may result in a detectable change in the K isotope composition of blood, which could serve as a biomarker (diagnostic indicator) of brain dyshomeostasis and AD. The K isotopes are thus a promising means to constrain or identify the underlying reasons for concentration differences between females and males. Although mouse organs display distinct elemental concentrations and isotopic compositions, there is no observed correlation between K concentration and isotopes in the same organs (Fig. 10). Therefore, K concentration does not have a significant effect on the isotopic distribution between different organs.

Sex and genetic background have minor effect on K isotopic composition

The female and male mice have large K isotope differences in brain and kidney, and their RBC and liver are comparable (Fig. 5). Previous studies revealed that sex did not affect Fe and Zn isotopic distribution in human blood and tissue organs.^{45,63,64} The K isotope measurements in mice in this study suggest that female organs are enriched in heavy K isotopes relative to male ones (Fig. 5), which is consistent with their Cu isotope differences between females and males,¹⁵ while such a difference was not observed for Zn isotopes.^{6,15}

The K concentration of different organs does not show a regular variation between female and male mice (Fig. 8). The feature is inconsistent with Fe and Cu, with the same trend of elemen-

tal concentration and isotopic composition between females and males.^{15,65} Given that the K isotopic difference in sex is true for each organ and RBC, suggesting that the difference might be controlled by a difference in K excretion and reabsorption between the sexes, and is then reciprocated to organs in the body, and further, there is an up-regulated K carrier in females compared to males. In general, sex does not have a major effect on K isotopic distribution between different organs of mice.

As shown in the result section about the genetic background, there is no statistically significant difference in the K isotopic compositions in any organs between the three distinct genetic background mice (P -value > 0.05; Fig. 4). The K isotopic differences between organs are much more important than those between genetic backgrounds. Overall, the genetic background has a limited effect on the K isotopic compositions of the different organs.

Conclusion

A span of nearly 2‰ K isotopic composition was observed between RBC, brain, liver, and kidney of ten 3-month-old mice, with a minor effect of genetic background and sex. The isotopic variations could be explained by different bonding environments of K in different organs and blood, making K isotopes a potential tool for tracing homeostatic changes associated with various diseases. The large fractionation and good correlation of K isotopes between RBC and brain suggest an effect of the K budget in the brain on blood, highlighting K isotopes as potential markers of chronic diseases such as hypertension, hyperkalemia, and cardiac diseases. These K-isotopic features in the brain hold promise for tracing neurodegenerative diseases. In summary, the K isotopic

distribution in organs and blood implies a sensitivity of K isotopes to specific biological processes or physiological mechanisms. The biomedicine of K isotopes is a promising research field worthy of further exploration.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

The data underlying this article are available in the article.

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