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Bodily variability of zinc natural isotope abundances in sheep

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Evidence is growing that the range of zinc stable isotope compositions, represented by the deviation of ^{66}Zn in permil units relative to a standard and expressed as δ^{66}Zn, is larger in organic matter than in inorganic material. This study reports the variations of δ^{66}Zn in various organs of sheep raised on a controlled diet. Zinc was purified by anion-exchange chromatography. The Zn concentrations and Zn stable isotope compositions were determined by quadrupole inductively coupled plasma mass spectrometry and multi-collector inductively coupled plasma mass spectrometry, respectively. The data show that δ^{66}Zn variability exceeds 1‰, with bone, muscle, serum and urine enriched in the heavy isotopes, and feces, red blood cells, kidney and liver enriched in light isotopes, all relative to the diet value. The ^{66}Zn enrichment of the circulating serum reservoir is likely to take place in the digestive tract, probably through the preferential binding of lighter isotopes with phytic acid, which is known to control the uptake of metallic elements. Mass balance calculations suggest that the ^{66}Zn depletion between diet and feces, which is not balanced by any other outward flux, leads to a secular isotopic drift in serum. A simple time-dependent two-box model, involving the gastro-intestinal tract on the one hand and the muscle and bone on the other, predicts that the maximum ^{66}Zn enrichment, which equals the difference in δ^{66}Zn between diet and bulk (~0.25‰), is reached after about ten years. Therefore, a better understanding of the variations of natural abundance of Zn isotopes in animals and humans will probably bring new perspectives for the assessment of their Zn status.

Zinc is an essential element for living organisms as it is required for the activity of >300 enzymes.[1] It has five stable isotopes, ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn and ^{70}Zn, with average natural abundances of 48.6, 27.9, 4.1, 18.8 and 0.6%, respectively. Although still at a very early stage, studies of the variations of Zn stable isotope ratios[a] in vegetal tissues show δ^{66}Zn values ranging from −0.6‰ to 1.4‰ in plants,[2–4] exceeding the variability known for geological terrestrial materials (~ −0.2‰ to 0.6‰; for reviews, see[5,6]). Such a large variation in δ^{66}Zn values is thought to be the result of mass-dependent fractionation during diffusion processes, first from growth media to plant, and then among plant physiological components (seed, stem and leaf). There is little data available for δ^{66}Zn values in animal tissues. Maréchal *et al.*[7] report values for standard bulk plankton (CRM 414), lobster liver (CRM TORT-2) and mussel tissue (CRM 278) that are ^{66}Zn-enriched by 0.07‰, 0.16‰ and 0.47‰, respectively, relative to English Channel seawater (0.35 ± 0.08‰[8]). Values of 0.50 ± 0.03‰ for standard bovine muscle (SRM 8414) and bovine liver (SRM 1577a) were reported by Ohno *et al.*[9] An average δ^{66}Zn value of 0.40 ± 0.15‰ (n = 9) can be calculated for human blood using data published by Maréchal *et al.*,[7] Stenberg *et al.*,[10] and Ohno *et al.*[9] Human hair is ^{66}Zn-depleted relative to blood as shown by three δ^{66}Zn values of -0.46 ± 0.04‰ and 0.07 ± 0.03‰[10] and −0.16‰.[9] The above preliminary data suggest that biological activity fractionates Zn isotopes in the body among organs. Therefore, natural Zn isotope variations are a potential source of novel information on the metabolic processing of Zn, which so far has only been investigated with isotopic techniques involving the addition of isotopically enriched tracers to food (e.g.[11]).

In the present study, we report Zn stable isotope compositions and concentrations for various organs including bone apatite, muscle, red blood cells (RBC), serum, liver and kidney of sheep raised under experimentally controlled

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[a] $\delta^{x}Zn = [(^{x}Zn/^{64}Zn)_{sample}/(^{x}Zn/^{64}Zn)_{standard} - 1].10^{3}$ and x = 66, 67 or 68). All δ^{66}Zn values in the text are with respect to the Lyon JMC 3-0749 L standard. All uncertainties cited are 2σ unless otherwise stated.

conditions. We also report Zn isotope compositions and concentrations for diet, feces and urine and propose a simple box model that allows the evolution of the Zn status with time to be predicted for herbivorous mammals.

EXPERIMENTAL

Animals and experimental design

The design of the feeding experiment for sheep is fully described in Zazzo *et al.*[12] A brief summary is given here. Four Suffolk cross lambs (2 males and 2 females), born at the Teagasc Production Research Centre, Athenry, Co. Galway, Ireland between March and April 2006, were taken from their mothers at pasture 0 to 7 days after birth and raised on artificial milk for 6 weeks. During this period, the animals were slowly weaned from the artificial milk and introduced to a commercial diet (pre-experimental diet). The animals were moved to the Teagasc Grange Beef Research Centre, Dunsany, Co. Meath, Ireland, in June 2006 but were maintained on the pre-experimental diet until the start of the experiment in September 2006. At the beginning of the experiment, the animals were switched to an experimental diet, consisting of 76% (wet weight basis) pelleted maize concentrate produced in one batch at Teagasc Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland, and 24% (wet weight basis) maize silage. The animals were assigned to two groups of one male and one female animal at either a low-energy allowance (LEA) or a high-energy allowance (HEA) of the experimental diet. Feed allowances were adjusted regularly throughout the experiment to ensure a constant weight gain of $50\,g.day^{-1}$ for animals receiving the LEA (# 9125 and #9169) and of $150\,g.day^{-1}$ for animals receiving the HEA (#9351 and #9646) (Table 1). Food was given to each animal individually in one batch in the morning together with unlimited access to tap water. The animals were kept on the experimental diet for 231 days. Upon completion of the experiment (3 April, 2007), they were transported to the Teagasc, Ashtown Food Research Center, Co. Dublin, Ireland, 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. Urine was collected from the bladder and acidified with a few drops of concentrated HCl. Bone aliquots were sampled in the jawbone around to the third molar socket area. 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.

Sample dissolution and Zn separation

Samples were dissolved in a 1:1 mixture of sub-boiled distilled concentrated HNO_3 and 30% H_2O_2 (analytical grade), evaporated to dryness, and re-dissolved in 1 mL of 7 N HCl + 0.001% H_2O_2. A 50 μL aliquot was taken for Zn concentration measurements and the remaining sample solution was processed for Zn isotope analysis. Zn was purified by anion-exchange chromatography using procedures adapted from Maréchal and Albarède[13] and Moynier *et al.*[14] Briefly, prior to each elution, 1.6 mL of macroporous resin AGMP-1 (100–200 mesh) is cleaned three times with 0.5 N HNO_3 alternating with H_2O, and conditioned by 6 mL of 7 N HCl + 0.001% H_2O_2. Matrix and Cu are removed using 30 mL of 7 N HCl + 0.001% H_2O_2. Fe is removed using 10 mL of 2 N HCl and Zn is finally eluted with 10 mL of 0.5 HNO_3. This solution is evaporated to dryness and re-dissolved in 1 mL of 1.5 N HBr. Zn is further purified on 0.5 mL of AG-1x8 resin (200–400 mesh) using 3 mL of 0.5 N HNO_3.

Mass spectrometry

The Zn isotopic ratios were measured by multi-collector inductively coupled plasma mass spectrometry (MCICPMS) at ENS-Lyon, using a Nu plasma 500 HR double-focusing mass spectrometer (Nu Instrument, Wrexham, UK) equipped with 12 Faraday detectors. The operating conditions were: rf power (1300 W), plasma gas flow rate (13 L/min) and auxiliary gas flow rate (0.9 L/min). Zn isotopes (M = 64, 66, 67 and 68) were measured in collectors H4, H2, Axial and L3. ^{63}Cu and ^{65}Cu were measured in collectors L2 and L4, respectively, and ^{62}Ni was measured in collector L5 in order to correct for the Ni contribution at mass 64 using the Ni natural abundance. Peak intensities were measured in Faraday detectors in static mode with a spectral resolution of $M/\Delta M = 1000$. Instrumental mass fractionation was corrected using Cu-doping (Cu SRM 976, National Institute of Standards and Technology, Bethesda, MD, USA) and standard-sample bracketing (Zn JMC 3-0749L, Johnson Matthey, Royston, UK) following the recommendations provided in Marechal *et al.*[15] and Albarède and Beard.[16] Sample measurement solutions were diluted to match the concentration of the standard mixture (Zn 0.5 ppm–Cu 0.5ppm). The external reproducibility (2σ) based on repeated measurements of the Zn JMC 3-0749L standard (n = 241) was 35 ± 28 ppm, 116 ± 94 ppm and 66 ± 56 ppm for the $^{66}Zn/^{64}Zn$, $^{67}Zn/^{64}Zn$ and $^{68}Zn/^{64}Zn$ ratio, respectively.

Zn concentrations were measured by quadrupole inductively coupled plasma mass spectrometry (QICPMS) at ENS-Lyon using a 7500 CX quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The following operating parameters of QICPMS were optimized: rf power (1550 W), plasma gas flow rate (15 L/min), auxiliary gas flow rate (2 L/min), carrier gas flow rate (0.9 L/min), makeup gas flow rate (0.15 L/min). The performance of the QICPMS instrument was optimized and checked daily by testing a standard mixture containing 1 ng/mL Li, Y, Co, Ce, and Tl in 3% HNO_3. Indium at 1 ng/mL was used as an internal standard to correct for any long-term instrumental drift.

RESULTS AND DISCUSSION

We expect, as a first approximation, that in a system I composed of three isotopes of mass x, y and z (z is the reference isotope) undergoing mass-dependent fractionation, $\delta^x I/\Delta x \approx \delta^y I/\Delta y$, where $\Delta x = x - z$ and $\Delta y = y - z$. Therefore, $\delta^{67}Zn$ and $\delta^{68}Zn$ should equal $1.5 * \delta^{66}Zn$ and $2.0 * \delta^{66}Zn$, respectively. The data fall onto a line of slope 1.56 and 1.97 for $\delta^{67}Zn$ and $\delta^{68}Zn$, respectively, showing that all the Zn isotope compositions are normally mass-dependent fractionated (Fig. 1).

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Table 1. Zn concentrations and isotope compositions for diet, organs, feces and urine of experimental sheep

#Sheep	# Chem	δ^{66}Zn ‰	±σ	δ^{67}Zn ‰	±σ	δ^{68}Zn ‰	±σ	[Zn] μg/g
Diet								
		0.30		0.41		0.54		
Mixture 01/08	01/08M	0.25		0.39		0.47		
		0.26		0.44		0.49		
		0.27	*0.03*	*0.41*	*0.03*	*0.50*	*0.03*	118
		0.32		0.42		0.60		
Mixture 28/08	28/08M	0.29		0.52		0.55		
		0.30		0.46		0.53		
		0.30	*0.02*	*0.47*	*0.05*	0.56	*0.04*	154
Mixture 12/12	12/12M	0.23		0.23		0.35		159
Bone								
9125	1B	0.43		0.64		0.86		
		0.43		0.52		0.78		
		0.43	*0.00*	*0.58*	*0.09*	*0.82*	0.06	78
		0.51		0.59		0.89		
9169	2B	0.46		0.64		0.85		
		0.48	*0.04*	0.62	*0.04*	*0.87*	*0.03*	62
9351	3B	0.34		0.46		0.62		
		0.39		0.36		0.71		
		0.36	*0.04*	*0.41*	*0.07*	*0.66*	*0.06*	77
		0.54		0.77		0.99		
9646	4B	0.53		0.63		0.97		
		0.53	*0.01*	*0.70*	*0.10*	*0.98*	*0.02*	78
Red blood cells								
9125	1H	−0.02		−0.09		−0.02		32
9169	2H	−0.13		−0.17		−0.23		36
9351	3H	−0.01		0.01		−0.11		64
		−0.07		−0.32		−0.23		
9646	4H	−0.11		−0.32		−0.20		
		−0.09	*0.03*	*−0.32*	*0.00*	*−0.22*	*0.02*	58
Serum								
9125	1S	0.46		0.69		0.91		26
9169	2S	0.57		0.94		1.18		26
9351	3S	0.41		0.72		0.81		23
9646	4S	0.52		0.60		0.99		30
Liver								
9125	1L	−0.34		−0.65		−0.71		62
9169	2L	−0.38		−0.68		−0.76		57
9351	3L	−0.65		−1.07		−1.39		
		−0.69		−1.08		−1.35		
		−0.67	*0.03*	*−1.08*	*0.01*	*−1.37*	*0.03*	80
9646	4L	−0.41		−0.86		−0.82		
		−0.36		−0.67		−0.76		
		−0.39	*0.03*	*−0.77*	*0.13*	*−0.79*	*0.04*	73
Kidney								
9125	1K	−0.02		−0.08		−0.01		127
		0.05		0.09		0.07		
9169	2K	0.00		−0.03		−0.03		
		0.05		−0.07		0.09		
		0.03	*0.03*	*0.00*	*0.08*	*0.04*	*0.06*	79
9351	3K	−0.37		−0.65		−0.85		
		−0.35		−0.54		−0.74		
		−0.36	*0.01*	*−0.59*	*0.08*	*−0.79*	*0.08*	142
9646	4K	−0.09		−0.37		−0.11		106
Muscle								
9125	1M	0.46		0.62		0.90		50
9169	2M	0.52		0.68		1.03		45
9351	3M	0.26		0.29		0.50		
		0.25		0.27		0.42		
		0.26	*0.01*	*0.28*	*0.02*	0.46	*0.06*	38
9646	4M	0.59		0.86		1.17		56
Feces								
9125	1C	0.14		0.16		0.25		
		0.15		0.23		0.15		
		0.15	*0.01*	*0.20*	*0.05*	*0.20*	*0.07*	741
		0.17		0.18		0.26		
9169	2C	0.15		0.01		0.21		

(*Continues*)

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Table 1. (Continued)

#Sheep	# Chem	δ^{66}Zn ‰	$\pm\sigma$	δ^{67}Zn ‰	$\pm\sigma$	δ^{68}Zn ‰	$\pm\sigma$	[Zn] µg/g
		0.16	*0.02*	*0.09*	*0.12*	*0.23*	*0.03*	799
		0.16		0.21		0.29		
9646	4C	0.21		0.08		0.30		
		0.20		0.31		0.27		
		0.19	*0.03*	*0.20*	*0.12*	*0.29*	*0.01*	712
Urine								
9125	1U	0.40		0.68		0.69		
		0.45		0.63		0.80		
		0.42	*0.04*	0.65	*0.03*	*0.74*	*0.08*	0.42
9172	5U	0.43		0.77		0.80		
		0.44		0.60		0.76		
		0.43	*0.01*	0.68	*0.12*	*0.78*	*0.02*	0.70
9414	6U	0.18		0.10		0.28		
		0.16		0.32		0.22		
		0.17	*0.02*	*0.21*	*0.15*	*0.25*	*0.04*	0.40
9443	7U	0.47		0.67		0.91		
		0.47		0.65		0.89		
		0.47	*0.00*	0.66	*0.02*	*0.90*	*0.02*	0.35

Bodily Zn isotope systematics

The Zn isotope data are given in Table 1. Seven replicates of three samples of the dietary ration give a δ^{66}Zn average value of 0.28 ± 0.06‰. The overall variability of the Zn isotope compositions in the organs of a single individual is nearly 1‰. Bone, muscle, serum and urine are ^{66}Zn-enriched by about 0.25‰ relative to the diet value, whereas feces, RBC, kidney and liver are ^{66}Zn-depleted by about 0.25, 0.35, 0.45 and 0.65‰, respectively (Fig. 2). A non parametric Kruskal-Wallis test has been performed to test whether the medians of the zinc isotope compositions of any two organs are statistically different. The results are reported in Table 2

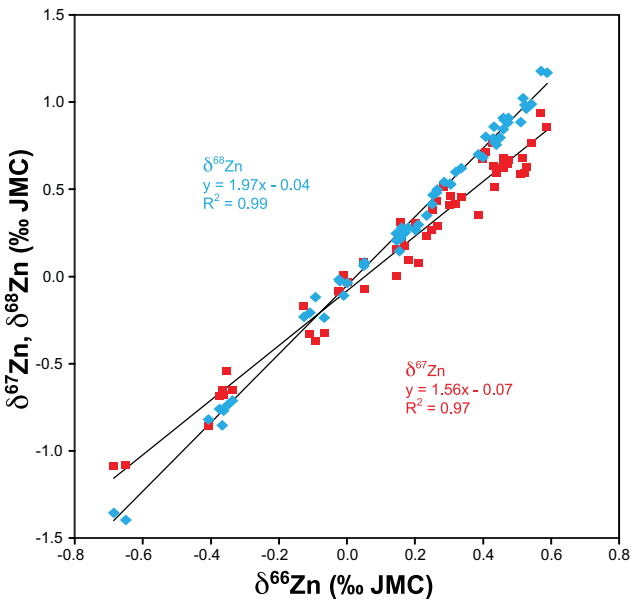

Figure 1. δ^{67}Zn vs. δ^{66}Zn and δ^{68}Zn vs. δ^{66}Zn. All the samples fall on the mass-dependent fractionation lines given by the theoretical relationships $\delta^{67}\text{Zn} = 1.5 * \delta^{66}\text{Zn}$ and $\delta^{68}\text{Zn} = 2.0 * \delta^{66}\text{Zn}$.

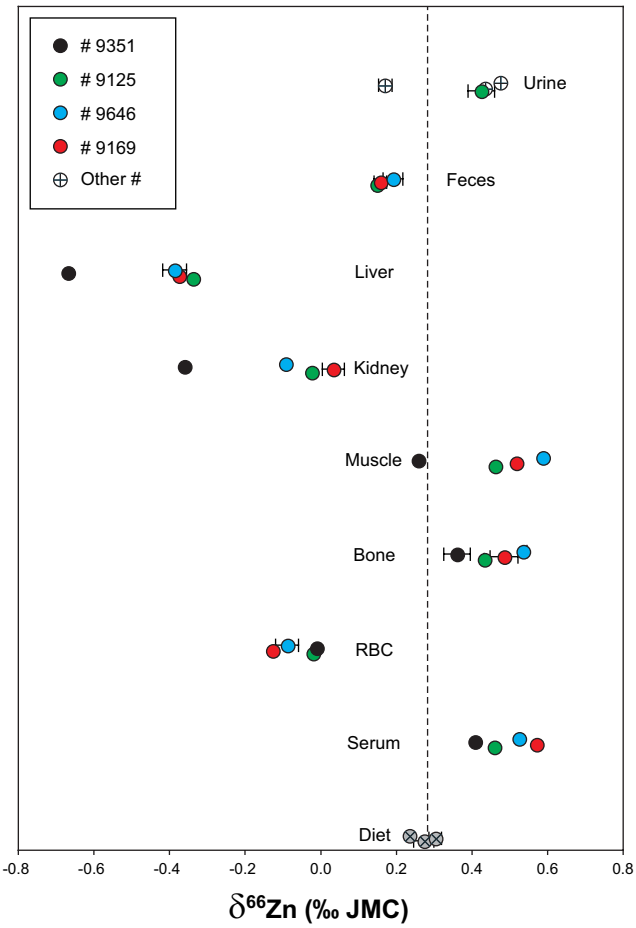

Figure 2. δ^{66}Zn values for the different organs of sheep. Errors bars are the 2σ external reproducibility of the JMC 3-0749L standard. Other sheep contributing to the urine data were slaughtered 10 weeks before the normal completion of the experiment.

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Table 2. H results for a Kruskal-Wallis test performed between pairs of organs or fluids

	Diet	Bone	RBC	Serum	Liver	Kidney	Muscle	Feces	Urine
Diet	—	10.54**	8.11*	7.03*	9.02*	9.84*	1.50	9.84*	2.63
Bone		—	8.60*	0.59	9.62*	10.54**	0.05	10.54**	0.90
RBC			—	8.63*	7.50*	0.24	6.82*	8.11*	8.60*
Serum				—	9.55*	7.03*	0.24	7.03*	2.34
Liver					—	6.63*	7.50*	9.02*	9.62*
Kidney						—	8.10*	9.84*	10.54**
Muscle							—	8.11*	0.77
Feces								—	6.82*
Urine									—

Data are treated unpaired since all sheep were raised on the same diet and therefore should be undifferentiated. Under the null hypothesis of equal population medians, H is approximately distributed according to a χ^2 distribution with k – 1 degrees of freedom and α level of significance. For two populations (k = 1) and a level of significance of 5% (a = 0.05), χ^2 equals 3.84. * is for significant results, i.e. when $P < 0.05$, and ** for is highly significant results, i.e. for $P < 0.0012$ after the Bonferroni correction for making 36 comparisons.

and show that the zinc isotope compositions of serum, bone, muscle and urine (group 1) are not statistically different. This pattern also holds for kidney and RBC (group 2). However, the zinc isotope compositions of diet, feces and liver are distinct from each other and from group 1 and group 2. However, the difference between the medians of kidney and liver or muscle and diet are small; the H result of the Mann-Whitney test is close to the χ^2 distribution (Table 2). This is because the δ^{66}Zn values of sheep #9351 are highly depleted in the heavy isotopes for muscle, kidney and liver with respect to the other sheep (Fig. 2). This animal, which was apparently free of abnormal behavior patterns and disease, also had the lowest δ^{66}Zn value for bone and serum, yet its balance between Zn uptake and loss was identical to that of the other sheep (Table 3). Zazzo *et al.*[12] showed that the carbon isotope turnover of sheep #9351 was similar to that of the other animals. At this stage, we have no explanation as to why the Zn isotopic distribution in the body of sheep #9351 differs from that observed in the other sheep.

The zinc concentrations were measured together with the Zn isotopic ratios (Table 1). All the values for the organs are tightly clustered in a concentration vs. isotope ratio diagram (Fig. 3). The 1/[Zn] vs. δ^{66}Zn distribution can be described as follows. Zn is supplied from serum, the circulating reservoir, to bone and muscle, which account for >90% of total body Zn,[19] without dilution effects or isotopic fractionation. The zinc concentrations and isotope compositions are correlated between serum, RBC and liver. This observation is meaningful because the Zn in RBC is bound to metallothioneins which are synthesized in the liver.[20,21] Mass balance calculation indicates that 60% of the Zn in RBC comes from liver, suggesting, therefore, that hepatic synthesis of metallothioneins utilizes as much as 40% of the Zn coming from the circulating pool. The zinc concentrations and isotope compositions are also correlated between diet, which is the sole inward flux, feces, the main outward flux, and serum. In this case, mass balance calculations indicate that the dietary Zn pool is decomposed into feces and serum in proportions of about 3/4 and 1/4, respectively. In physiological terms, this means that at steady state, i.e. when the loss balances the intake, feces represents ~75% of the total Zn losses, an estimate in agreement with metabolic data on humans[19] and animals.[22] The site of the isotopic fractionation between diet and serum is unknown, but it probably occurs in the digestive tract. Two mechanisms, which can act synergistically, can explain this heterogeneous isotope distribution. The first is the presence of inhibitors of Zn absorption in diet containing plant-based foodstuffs. The best known inhibitor of metal absorption is phytate (inositol hexakisphosphate), which is the principal storage form of

Table 3. Zn balance in sheep. Total output is calculated by the sum of fecal, urine and wool output. Intake is calculated as the sum of dry matter (DM) intake of concentrate and silage

Specimen #ID	#9125*	#9169*	#9351**	#9646**
Concentrate Intake				
g/day	920	680	1555	1860
g DM/day	768	568	1298	1553
g digestible DM/day	631	467	1067	1277
g DM loss/day	137	101	231	276
Silage Intake				
g/day	295	220	500	600
g DM/day	82	61	139	167
g digestible DM/day	61	45	103	123
g DM loss/day	22	16	37	44
Body Weight (kg)	59.3	47.2	70.6	80.4
Urine (ml/day)#	1253	1039	1446	1608
Wool (g/day)§	12	10	13	15
Diet [Zn] (μg/g)	140	140	140	140
Feces [Zn] (μg/g)	741	799	751	712
Urine [Zn] (μg/ml)	0.5	0.5	0.5	0.5
Wool [Zn] ((μg/g)¶	150	150	150	150
Zn intake (mg/day)	119	88	201	241
Zn urine output (mg/day)	0.6	0.5	0.7	0.8
Zn fecal output (mg/day)	117	94	201	228
Zn wool output (mg/day)	1.8	1.5	2.0	2.2
Balance (mg Zn/day)	**−0.6**	**−7.6**	**−2.5**	**9.8**
Balance (%)	**−0.5**	**−8.6**	**−1.2**	**4.1**

* Low energy allowance.
** High energy allowance.
# Urine production (Up, 10^{-4} ml/s) is calculated using the relationship with body weight (BW, kg), $Up = 5.1BW^{0.82}$,[17]
§ Wool production is calculated using a daily wool growth value of 0.7 mg/cm2/day ([18]) and the relationship between body surface area (S, m^2) and body weight (BW, kg), $S = 0.11BW^{0.67}$,[17]
¶ Average value[19]
#9125 and #9351 are males and #9169 and #9646 are females.

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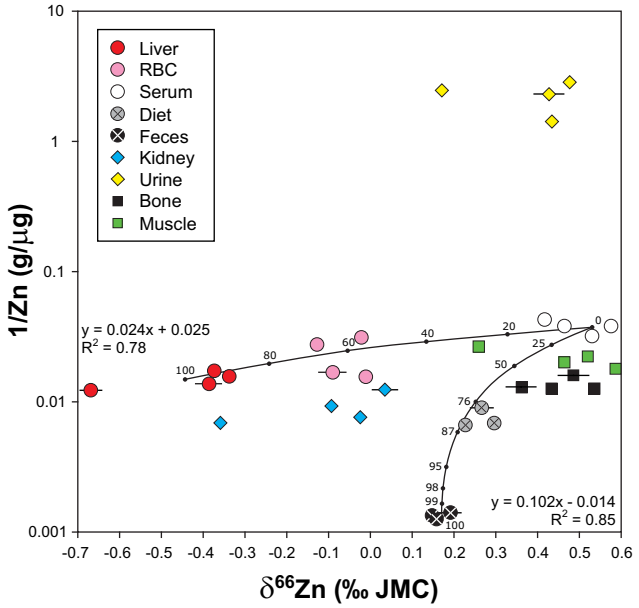

Figure 3. δ^{66}Zn vs. 1/Zn for the different organs of sheep. Numbers indicate the proportion (%) of the end-members for a given mixing line. Errors bars are the 2σ external reproducibility of the JMC 3-0749L standard.

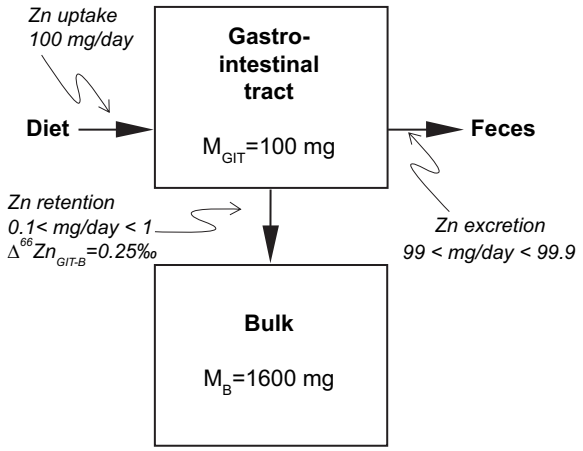

Figure 4. Schematic diagram of a two-box model for Zn isotopes in mammals. No flux was set between the Gastro-Intestinal Tract (**GIT**) and the Bulk (**B**) reservoirs in order to obtain a net Zn retention in **B**. The Zn retention flux is associated with a ^{66}Zn-enrichment corresponding to the measured difference between bone/muscle and diet (0.25‰). All initial δ^{66}Zn values equal 0‰. The value of the Zn uptake is set at 100 mg, which corresponds to the Zn requirement for a minimal growth rate of sheep. The value of Zn excretion is calculated for a zero Zn balance in **GIT** given an arbitrary value of Zn retention of 0.1, 0.25, 0.5 or 1 mg/day. The mass of Zn in **GIT** equals the Zn uptake in order to lead to a daily total draining of **GIT**. The mass of Zn in **B** is calculated for a body mass of 55 kg given that a human body of 70 kg contains approximately 2 g of Zn.

phosphorus in plant tissues, especially bran and seeds.[23] Phytase is produced by rumen microorganisms, and it allows ruminants to partly utilize phytate. The partial release of the dietary Zn into the lumen might be associated with isotope fractionation processes due to incomplete reaction, but the breakdown of phytate, an issue of great complexity that depends on the catalytic efficiency of phytase and the presence of other cations in the medium,[24–26] does not allow us to predict which isotope will be preferentially affected. The second mechanism involves Zn transporters. It has recently been recognized that Zn uptake kinetics are well described by active processes that involve transporter(s), which facilitate the passage of Zn across the enterocyte and the basolateral membrane into the portal circulation.[27] Zinc isotope preferential binding to transporters is also likely to occur in this case, and this could explain the isotopic difference between diet and serum. However, the heterogeneous distribution of Zn isotopes among organs and body fluids reflects the complexation of this metal by strong ligands such as the thiol group of cysteine which form aspartate transcarbamylase in the cytoplasm and the imidazole of histidine involved in degradation and digestion processes in the extracellular matrix.[28] The role of ZnT proteins in Zn homeostasis is also particularly complex.[29] Therefore, the formation of complexes with a broad range of bond strengths, as well as changes in Zn coordination from 4-, 5- to 6-fold, may, as predicted for iron, induce isotopic fractionation.[30]

A δ^{66}Zn drift in ageing herbivores?

In this study, we found that the bulk (97%) of Zn output occurs through the feces (Table 3). These values are consistent with those reported for sheep,[31] lambs[32] and cattle,[33] and seem to be characteristic of ruminant animals. A mass balance involving feces and urine Zn concentrations, and isotope ratios shows that the total δ^{66}Zn output is lacking 0.12‰ relative to the diet value. Wool accounts for a very small fraction (2%) of the Zn output (Table 3), and is unlikely to balance the isotope output because it would require an unrealistic wool δ^{66}Zn value of more than 5‰. Therefore, mass conservation implies that the excretion of ^{66}Zn-depleted feces must be balanced by a ^{66}Zn-enrichment in the body. We used a simple two-reservoir model to predict the evolution of the body's bulk Zn isotope composition as a function of time (Fig. 4). In this model, the gastro-intestinal tract is supplied daily by diet and provides Zn irreversibly to the bulk (bone/muscle) reservoir. This flux is associated with a ^{66}Zn-enrichment corresponding to the measured difference between bone/muscle and diet (0.25‰). The rate of change of the moles of ^{64}Zn and ^{66}Zn in the gastro-intestinal tract (g) is given by:

$$\partial^{64}\mathrm{Zn_g}/\partial t = {}^{64}J_\mathrm{d} - {}^{64}J_\mathrm{b} - {}^{64}J_\mathrm{f} \quad (1)$$

$$\partial^{66}\mathrm{Zn_g}/\partial t = {}^{66}J_\mathrm{d} - {}^{66}J_\mathrm{b} - {}^{66}J_\mathrm{f} \quad (2)$$

where $^{64}J_\mathrm{d}$, $^{64}J_\mathrm{b}$ and $^{64}J_\mathrm{f}$, and $^{66}J_\mathrm{d}$, $^{66}J_\mathrm{b}$ and $^{66}J_\mathrm{f}$ are the fluxes of ^{64}Zn and ^{66}Zn from diet (d) toward bulk (b) and feces (f), respectively. The full set of calculations is given as Supplementary Equations (see Supporting Information).

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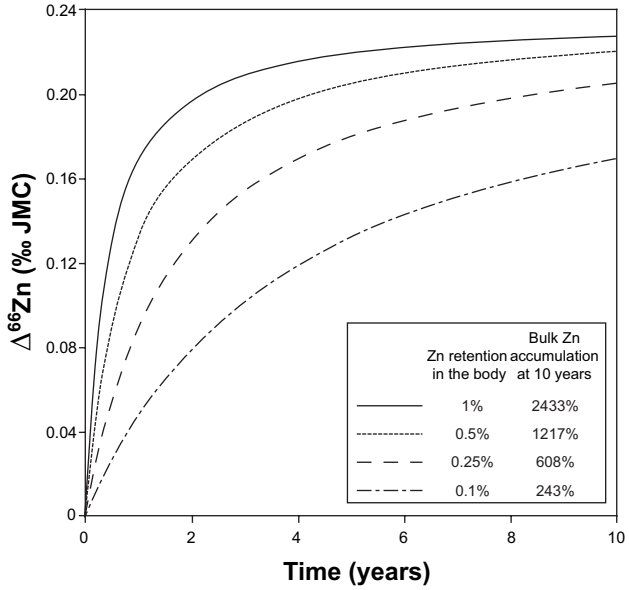

Figure 5. Evolution over 10 years of the computed ^{66}Zn-enrichment of a herbivore's Zn bulk value. Each line represents a given proportion of Zn retention (mg/day) in the body. The corresponding bulk Zn accumulation at 10 years is indicated.

At steady-state conditions, the analytical solution is:

$$\frac{^{64}J_b}{^{64}J_f} = \frac{\delta^{66}Zn_g - \delta^{66}Zn_d}{\delta^{66}Zn_d - \delta^{66}Zn_b} \quad (3)$$

Equation (3) allows us to calculate a $\delta^{66}Zn_b$ steady-state value of ~0.25‰ with $\delta^{66}Zn_d$ and $\delta^{66}Zn_g$ values of 0‰ (Supplementary Equations, see Supporting Information). The time-dependent differential system of Eqns. (1) and (2) was also integrated numerically. The evolution with time of the difference between $\delta^{66}Zn_d$ and $\delta^{66}Zn_b$ ($\Delta^{66}Zn_{d-b}$) is shown in Fig. 5. The evolution with time of $\Delta^{66}Zn_{d-b}$ is calculated for arbitrary values of Zn retention efficiencies (i.e. the irreversible supply of Zn to the bulk), giving bulk Zn accumulation at 10 years (i.e. body growth) of between 243% and 2430%. The results of the modeling indicate that for a reasonable growth rate of ~200% at 10 years, the body's bulk Zn isotope composition can be enriched by <0.2‰ relative to the diet with a Zn retention of 0.1%. However, the most elegant demonstration of the existence of a Zn isotope drift would be to measure the $\delta^{66}Zn$ value in biopsies taken at regular intervals during the lifetimes of experimental mammals.

CONCLUDING REMARKS

Using MCICPMS, we demonstrate that the variability of Zn isotope compositions among body parts and fluids in four sheep specimens exceeds 1‰, a range larger than that reported so far for terrestrial geological material. Large isotopic variations among organs and fluids of higher vertebrates have also been reported for calcium[34] (~2‰) and iron[35] (~4‰). For comparison, the typical terrestrial geological isotopic ranges are about 1‰ and 4‰ for calcium[36] and iron,[37] respectively. As for zinc, the range of the calcium and iron isotope compositions at the scale of a single organism therefore encompasses the terrestrial geological variability. Such an important isotopic difference between organs has opened interesting perspectives for revisiting the metabolism of calcium and iron in normal and pathological conditions.[38,39] It is likely that the present results on zinc isotope compositions will stimulate the study of zinc metabolism and perturbation of zinc status.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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