Earth and Planetary Science Letters 374 (2013) 164–172

Contents lists available at SciVerse ScienceDirect

Earth and Planetary Science Letters

journal homepage: www.elsevier.com/locate/epsl

Iron, copper and zinc isotopic fractionation up mammal trophic chains

Klervia Jaouen [a], Marie-Laure Pons [b], Vincent Balter [b,*]

[a] Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany
[b] Laboratoire de Géologie de Lyon UMR 5276 CNRS-Ecole Normale Supérieure de Lyon, 15, parvis René Descartes-BP 7000, 69342 Lyon Cedex 07, France

ARTICLE INFO

Article history:
Received 16 June 2012
Received in revised form
17 May 2013
Accepted 23 May 2013
Editor: J. Lynch-Stieglitz
Available online 19 June 2013

Keywords:
non-traditional isotopes
isotopic fractionation
trophic chain
paleoecology
paleodiet

ABSTRACT

There is a growing body of evidence that some non-traditional elements exhibit stable isotope compositions that are distinct in botanical and animal products, providing potential new tracers for diet reconstructions. Here, we present data for iron (Fe), copper (Cu) and zinc (Zn) stable isotope compositions in plants and bones of herbivores and carnivores. The samples come from trophic chains located in the Western Cape area and in the Kruger National Park in South Africa. The Fe, Cu and Zn isotope systematics are similar in both parks. However, local Cu, and possibly Zn, isotopic values of soils influence that of plants and of higher trophic levels. Between plants and bones of herbivores, the Zn isotope compositions are ^{66}Zn-enriched by about 0.8‰ whereas no significant trophic enrichment is observed for Fe and Cu. Between bones of herbivores and bones of carnivores, the Fe isotope compositions are ^{56}Fe-depleted by about 0.6‰, the Cu isotope compositions are ^{65}Cu-enriched by about 1.0‰, and the Zn isotope compositions are slightly ^{66}Zn-depleted by about 0.2‰. The isotopic distributions of the metals in the body partly explain the observed trophic isotopic systematics. However, it is also necessary to invoke differential intestinal metal absorption between herbivores and carnivores to account for the observed results. Further studies are necessary to fully understand how the Fe, Cu and Zn isotope values are regulated within the ecosystem's trophic levels, but the data already suggests significant potential as new paleodietary and paleoecological proxies.

1. Introduction

Iron (Fe), copper (Cu) and zinc (Zn) are three essential elements for life. These are bound to a variety of ligands to form metalloenzymes and are present under different redox states (Fe and Cu), leading an organism to have heterogeneous isotopic composition in the body. The range of variations of δ^{56}Fe, δ^{65}Cu and δ^{66}Zn[1] values within an organism, being vegetal or animal, is of similar amplitude than those reported so far for most geological terrestrial materials (δ^{56}Fe: −2.7‰ to +1‰ Johnson et al., 2003; Zhu et al., 2000; δ^{65}Cu −3‰ to +2.5‰, Larson et al., 2003; Markl et al., 2006; δ^{66}Zn: −0.5‰ to +1.4‰, Pons et al., 2011). In plants, the metal isotope compositions vary between seeds, stem and leaves, all these organs being isotopically different than the growth media (Weiss et al., 2005; Guelke and von Blanckenburg, 2007; Moynier et al., 2009; Weinstein et al., 2011; Jouvin et al., 2012). The overall variability reported so far for plants ranges from 0‰ to −1.6‰ for δ^{56}Fe (Guelke and von Blanckenburg, 2007; von Blanckenburg et al., 2009), from −1‰ to +0.4‰ for δ^{65}Cu (Weinstein et al., 2011; Jouvin et al., 2012), and from −0.6‰ to +1.3‰ for δ^{66}Zn (Weiss et al., 2005; Moynier et al., 2009). In animals, metal isotope compositions vary among organs (Walczyk and von Blanckenburg, 2002; Balter et al., 2010; Albarède et al., 2011; Hotz et al., 2011; Jaouen et al., 2012). For iron, the whole body is ^{56}Fe-depleted relative to the diet due to a strong isotopic fractionation during Fe intestinal absorption (Walczyk and von Blanckenburg, 2002). In experimental pigs, the δ^{56}Fe values range from −0.4‰ in liver to −1.8‰ in blood for a dietary δ^{56}Fe value of 0‰ (Hotz et al., 2011). In humans, the δ^{56}Fe values can range from −3.5‰ in hair and muscle (Walczyk and von Blanckenburg, 2002) to +0.4‰ in bone (Jaouen et al., 2012). Concerning Cu, the δ^{65}Cu values range from −1.5‰ in liver to +1.5‰ in kidney of experimental sheep and mice for a diet δ^{65}Cu value of 0‰ (Balter and Zazzo, 2011). Human blood is characterized through its constituents, serum and erythrocytes, by a range of the Cu isotope compositions of about 1.6‰, the lowest δ^{65}Cu value of serum being −0.7‰ and the highest δ^{65}Cu value of erythrocytes being +0.9‰ (Albarède et al., 2011). Bodily variations of the δ^{66}Zn values range from −0.5‰ in liver to +0.5‰ in bone, muscle and serum, for a diet δ^{66}Zn value of +0.2‰ (Balter et al., 2010). The spread of the δ^{66}Zn value is about 2‰ in humans, liver being characterized by negative value down to −1‰

* Corresponding author. Tel.: +33 472 728 488.
E-mail address: Vincent.Balter@ens-lyon.fr (V. Balter).

[1] $\delta^{65}Cu=[(^{65}Cu/^{63}Cu)_{sample}/(^{65}Cu/^{63}Cu)_{standard}-1]\times 10^3$. All δ^{65}Cu values in the text are with respect to the NIST-SRM 976 standard. $\delta^{x}Fe=[(^{x}Fe/^{54}Fe)_{sample}/(^{x}Fe/^{54}Fe)_{standard}-1]\times 10^3$and $x=56$ or 57. All δ^{x}Fe values in the text are with respect to the IRMM 14 standard. $\delta^{x}Zn=[(^{x}Zn/^{64}Zn)_{sample}/(^{x}Zn^{64}Zn)_{standard}-1]\times 10^3$and $x=66$, 67 or 68. All δ^{x}Zn values in the text are with respect to the JMC 3-0749L standard.

0012-821X/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.epsl.2013.05.037

and bone by positive values up to +1‰ (Albarède et al., 2011; Jaouen et al., 2012).

The above preliminary data suggest that biological activity heterogeneously redistributes metal isotopes in the organism, leading the different organs to be isotopically fractionated. The two major potential factors of the isotopic fractionation of metals are thought to be the oxidation state and the nature of ligands. Briefly, for metals bearing various oxidation states (as for Fe and Cu), oxidized compounds should be isotopically heavier than their reduced equivalent (Bigeleisen and Goeppert-Mayer, 1947). Zinc exists as Zn^{2+} solely, and Zn isotopic fractionation is expected to occur only during Zn exchange between ligands of distinct bond energies.

The existence of widespread isotopic fractionations of Fe, Cu and Zn between diet and animal organs, suggests that the metal isotope compositions can be used as potential dietary tracers in natural contexts. This has been previously suggested by Walczyk and von Blanckenburg (2005) concerning the Fe isotope compositions. In the present study, we report the results of Fe, Cu and Zn stable isotope compositions for plants and bones of herbivores and carnivores coming from two trophic chains in South Africa, the Kruger National Park (KNP) and Western Cape (WC).

2. Material and method

The sampling consists of fresh bones of herbivores and carnivores coming from the Kruger National Park (KNP) and Western Cape (WC), South Africa (Fig. 1). Also included are samples of plants debris that were found stuck in the dentine grooves of the occlusal surface in teeth of herbivores. The samples were collected at the Ditsong National Museum of Natural History (Pretoria, South Africa). Bone aliquots were sampled in jaws. All the samples belonging to protected species by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) were exported to France and re-exported to South Africa according to the international agreements between the French and the South African governments.

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 further redissolved in 1 mL of 7 N HCl+0.001% H_2O_2. The solution was processed for isotope analysis according to the technique of Maréchal et al. (1999), fully described in Maréchal and Albarède (2002). Using quartz column filled with the AG MP-1 anion-exchange resin (100–200 mesh, Bio-Rad), Cu was eluted with 20 mL of 7 N HCl+0.001% H_2O_2, Fe with 10 mL of 2 N HCl +0.001% H_2O_2, and Zn with 10 mL of 0.5 N HNO_3. This procedure constitutes the first method (thereafter labeled method *M*1) for extracting Zn from the matrix. A second method (thereafter labeled method *M*2) was also tested for comparative purposes. The starting solution is evaporated to dryness and re-dissolved in 1 mL of 1.5 N HBr, and Zn is further purified on 0.5 mL of AG-1x8 resin (200–400 mesh) using 3 mL of 0.5 N HNO_3. Whatever the procedure and the element, the chemical extraction was repeated once to get rid of residual trace elements.

Metal isotopic ratios were measured by multiple-collector inductively coupled plasma mass spectrometry (MC-ICPMS) at ENS-Lyon, using either a VG Plasma 54 (P54) or a Nu Plasma HR (Nu500) for Zn, a Nu500 for Cu, and a large radius Nu Plasma 1700 (Nu1700) for Fe. Analytical conditions for Zn isotopic ratios are provided in Maréchal et al. (1999) and in Balter et al. (2010) for measurements by means of the P54 and the Nu500 MC-ICPMS, respectively. In both cases, instrumental mass fractionation was corrected using Cu-doping (Cu NIST-SRM 976) and standard-sample bracketing (Zn JMC 3-0749L). Copper isotopic ratios were determined on the Nu500 by Zn-doping (Zn JMC 3-0749L) and standard-sample bracketing (Cu NIST-SRM 976). The Cu/Zn doping technique requires Cu and Zn isotope measurements using wet plasma due to large isotopic effects on the desolvator devices (Aridus and DSN). For Fe, isotopic ratios were run using dry plasma and instrumental mass fractionation was controlled by standard-sample bracketing (Fe IRMM14). Metal concentrations were measured at ENS-Lyon using an Agilent 7500CX quadrupole ICPMS.

Several in-house standards were routinely analyzed for the assessment of the precision. The results are shown in Table 1, and give a typical external reproducibility (2σ, where σ is the standard deviation of the results) of 65 ppm for Zn, 60 ppm for Cu and 110 ppm for Fe. Isotopic fractionation was mass dependent for Zn whatever the MC-ICPMS used for analysis (Fig. 2A and B), because the δ^{66}Zn vs. δ^{67}Zn values and the δ^{66}Zn vs. δ^{68}Zn values fall on mass fractionation lines close to the theoretical value of 1.5 and 2, respectively. The δ^{56}Fe vs. δ^{57}Fe values fall on a mass fractionation line of slope 1.54 (± 0.03) close to the theoretical value of 1.5 (Fig. 2C). In all cases, offsets are negligible (Fig. 2A–C).

3. Results

The isotopic and concentration results are presented in Table 2 and the isotopic results for Zn, Cu and Fe graphically represented as a function of sample type in Figs. 3, 4, and 5, respectively.

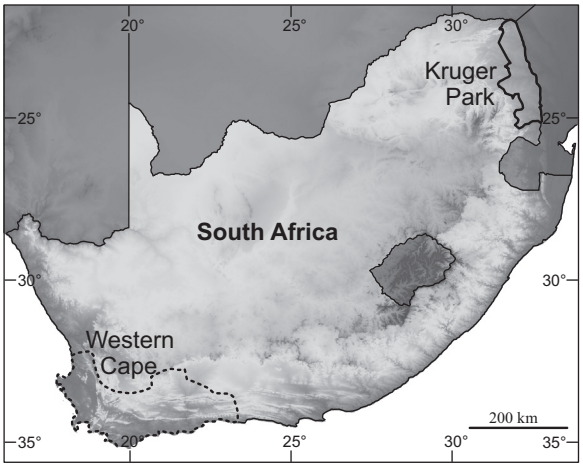

Fig. 1. Localization of the Kruger National Park (KNP) and Western Cape (WC) areas in South Africa.

Table 1
Zn, Cu and Fe isotopic values for the several in-house standards used during the course of the study. The external reproducibility is expressed as 2σ, where σ is the standard deviation of the results.

Sample	Mean (‰)	Min (‰)	Max (‰)	$\pm 2\sigma$	*n*
δ^{66}Zn					
ZnO (sheep muscle)	0.64	0.57	0.67	0.08	8
Zn49 (fossil bone)	0.65	0.64	0.67	0.02	7
3L (sheep liver)	−0.66	−0.72	−0.63	0.08	4
ZnST4 (soil)	0.36	0.28	0.41	0.08	6
δ^{65}Cu					
gb (copper coin)	0.14	0.10	0.20	0.04	16
CuO (sheep muscle)	−0.31	−0.36	−0.25	0.06	13
Cu49o (fossil bone)	−0.30	−0.25	−0.36	0.06	21
Cu49 (fossil bone)	−0.40	−0.49	−0.32	0.08	23
δ^{56}Fe					
3H (sheep blood)	−1.87	−2.10	−1.67	0.12	60
L1 (human blood)	−2.90	−3.01	−2.80	0.10	49

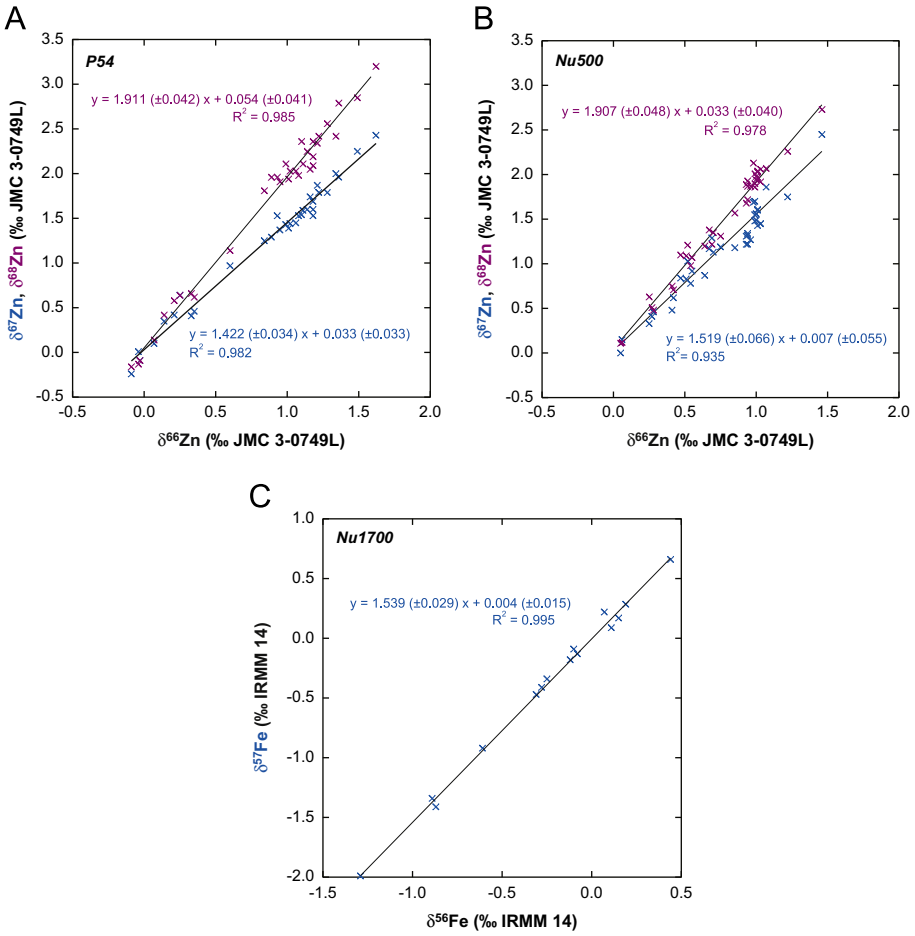

Fig. 2. Mass fractionation lines for Zn and Fe isotopes on the different MC-ICPMS used in the present study. Regressed slopes are similar within error to theoretical equilibrium mass-dependent fractionation. Scattering around the regression lines for Zn is partly due to the fact that several sessions were taken into account.

3.1. Zinc

For samples that have been replicated, the Zn isotope compositions are very close whatever the method used for purifying Zn from the matrix (*M*1 for HCl; *M*2 for HBr), and the type of MC-ICPMS used for analysis (P54 or Nu500). The comparison of results obtained using two different combinations of methods+analyses are possible for *M*1+Nu500 vs. *M*2+Nu500 and *M*1+Nu500 vs. *M*2 +P54. In the first type of comparison (*M*1+Nu500 vs. *M*2+Nu500), only the method is different, while in the second type of comparison (*M*1+Nu500 vs. *M*2+P54), both method and analyses are different. When comparing the methods, the external reproducibility (2σ) is on average 50 ppm for 5 samples that have been replicated into 14 aliquots (TM4403; TM38253; AZ1132; TM3059; TM3220). When comparing methods and analyses, the external reproducibility (2σ) is on average 112 ppm for 6 samples that have been replicated into 25 aliquots (TM4404; TM17003a; TM17003b; TM4081; TM16659PR; TM16690PR). Compared to the situation where only methods are different, the reproducibility is twice lower when both the methods and the analyses are different but the numbers remain fully acceptable. We therefore considered the whole dataset of Zn isotope compositions homogeneous whatever the methods and the analyses.

The average Zn concentration is 117 ± 95 μg/g and ranges from 8 to 335 μg/g in bones and is 74 ± 38 μg/g and ranges from 43 to 116 μg/g in plants. For bone, except the value at 8 μg/g, the variations of Zn concentration are in the range of natural variations for mammals, which is 50–400 μg/g (Jaouen et al., 2012). For plants, the concentrations do not exceed 200 μg/g, which seems to be a threshold toxic value (Balsberg Påhlsson, 1989). There is no patterning of Zn concentrations between carnivores, herbivores and plants, and the concentrations are not correlated to the isotopic compositions.

At KNP, the δ^{66}Zn values for plants ranges from −0.09‰ to 0.52‰ with an average value of 0.22 ± 0.37‰. Two δ^{66}Zn data for plant at WC gives an average value of 0.15 ± 0.14‰. The Zn isotope compositions bones of herbivores range from 0.84‰ to 1.62‰, with an average value of 1.18 ± 0.20 at KNP, and from 0.55‰ to 1.01‰, with an average value of 0.75 ± 0.18‰ at WC. At KNP, the Zn isotope compositions of the bones of carnivores range from 0.94 to 1.18, with a mean value of 0.95 ± 0.20. Statistical comparisons for the KNP samples show that the herbivores are significantly ^{66}Zn-enriched relative to plants (*t*-test, $P < 10^{-4}$), and that carnivores are slightly ^{66}Zn-depleted relative to herbivores (*t*-test, $P = 0.06$; Fig. 3). This Zn isotopic pattern holds for WC also, despite the smallest number of samples, the herbivores being significantly ^{66}Zn-enriched relative to plants (*t*-test, $P = 0.04$), and the unique carnivore analyzed so far being the lowest δ^{66}Zn value of all the animals.

3.2. Copper

The Cu concentrations range from 0.93 to 297 μg/g in bones and from 1.6 to 12 μg/g in plants. The typical range of Cu concentrations in mammal bones is the same than in plants, i.e. 1–50 μg/g (Burkhead et al., 2009; Jaouen et al., 2012). The Cu concentrations are not distinctive of a trophic position nor are they correlated to isotopic compositions. These first data, yet preliminary, clearly show

Table 2
Zn, Cu and Fe isotopic compositions and concentrations of the samples. Asterisks indicate concentration values outside the typical range.

Latin name	Common name	Area	Sample ID		δ^{66}Zn	$\pm 2\sigma$	δ^{67}Zn	$\pm 2\sigma$	δ^{68}Zn	$\pm 2\sigma$	[Zn]	δ^{65}Cu	$\pm 2\sigma$	[Cu]	δ^{56}Fe	$\pm 2\sigma$	δ^{57}Fe	$\pm 2\sigma$	[Fe]
			Museum	Chemistry	(‰)		(‰)		(‰)		(μg/g)	(‰)		(μg/g)	(‰)		(‰)		(μg/g)
Carnivores																			
Hyaena brunnea	Brown hyena	KNP	TM4404	ML10[a]	1.11		1.59		2.11										
				ML7[a]	1.14		1.60		2.25										
				ML9[a]	1.18		1.53		2.09										
				43Zn[b]	0.98		1.69		2.13										
				43Zn[b]	0.99		1.70		2.01										
					1.08	*0.18*	*1.62*	*0.15*	*2.12*	*0.17*	8*				−0.87		−1.41		1.6*
Panthera leo	Lion	KNP	TM4403	36Zn[b]	0.99		1.48		1.86										
				Zn8[c]	1.00		1.49		1.94										
				Zn9[c]	1.03		1.45		2.06										
				Zn9[c]	0.94		1.32		1.93										
					0.99	*0.08*	*1.43*	*0.16*	*1.95*	*0.16*	73	−0.14		10	−1.29		−1.99		273
Panthera leo	Lion	KNP	TM13924												−0.31		−0.47		
															−0.25		−0.34		
				Zn14[c]	0.94		1.22		1.87		87	0.19		31	*−0.28*	*0.04*	*−0.41*	*0.09*	311
Crocuta crocuta	Spotted hyena	KNP	TM19370	ML2[a]	1.06		1.45		2.03										
Panthera leo	Lion	WC	TM38253	Zn18[c]	0.51		0.83		1.09										
				42Zn[b]	0.54		0.78		0.98										
					0.52	*0.05*	*0.80*	*0.06*	*1.04*	*0.16*	146				−0.89		−1.34		167
					0.95														
Herbivores					0.20														
Hippotragus niger	Sable antelope	KNP	TM17003a	ML12[a]	1.01		1.39		1.94										
				ML12[a]	1.02		1.44		2.03										
				37Zn[b]	1.07		1.86		2.07										
					1.03	*0.06*	*1.56*	*0.52*	*2.01*	*0.13*	63				−0.61		−0.92		19
Hippotragus niger	Sable antelope	KNP	TM17003b	ML4[a]	1.22		1.79		2.42										
				37Zn−b[b]	1.22		1.75		2.26										
					1.22	*0.002*	*1.77*	*0.05*	*2.34*	*0.23*									
Hippotragus niger	Sable antelope	KNP	TM16999	45Zn[b]	0.85		1.18		1.57		83				0.44		0.66		18
Sylvicapra grimmia	Common duiker	KNP	TM4081	ML6[a]	0.95		1.37		1.91										
				ML6[a]	0.93		1.53		1.96										
				ML6bis[a]	0.84		1.25		1.81										
				ML6bis[a]	0.89		1.29		1.96										
				31Zn[b]	1.01		1.60		1.97										
				31Zn[b]	1.03		1.45		1.92										
				31Zn[b]	1.01		1.43		2.04			−1.20							
				31Zn[b]	0.99		1.55		1.90			−1.37							
					0.95	*0.13*	*1.44*	*0.25*	*1.93*	*0.13*	176	*−1.29*	*0.24*	0.93*					
Damaliscus lunatus	Topi	KNP	TM17007	ML1[a]	1.49		2.25		2.85										
				ML11[a]	1.36		1.96		2.79										
					1.42	*0.18*	*2.11*	*0.42*	*2.82*	*0.08*									
Equus burchellii	Zebra	KNP	AZ1132	35Zn[b]	0.94		1.34		1.72						−0.10		−0.09		126
				Zn1[c]	0.93		1.31		1.89										
					0.93	*0.01*	*1.32*	*0.04*	*1.80*	*0.25*		−0.68		36					
Equus burchellii	Zebra	KNP	TM16690	Zn15[c]	1.46		2.45		2.73										
Redunca arundinum	Southern reedbuck	KNP	TM16659	ML5[a]	1.28		1.79		2.56										
Syncerus caffer	Buffalo	KNP	TM17009	ML8[a]	1.18		1.69		2.36										
Aepyceros melampus	Impala	KNP	AZ2137	ML3[a]	1.62		2.43		3.20										
Aepyceros melampus		KNP	AZ2131	L1os[a]	0.99		1.43		2.11										
Aepyceros melampus		KNP	AZ2138	L2os[a]	1.18		1.60		2.19										
Aepyceros melampus		KNP	AZ2126	L3os[a]	1.21		1.87		2.34										
Aepyceros melampus		KNP	TM17667	L4[a]	1.10		1.54		2.36										
Aepyceros melampus		KNP	TM17686	L7[a]	1.34		2.00		2.42										

Table 2 (*continued*)

Latin name	Common name	Area	Sample ID		δ^{66}Zn	$\pm 2\sigma$	δ^{67}Zn	$\pm 2\sigma$	δ^{68}Zn	$\pm 2\sigma$	[Zn]	δ^{65}Cu	$\pm 2\sigma$	[Cu]	δ^{56}Fe	$\pm 2\sigma$	δ^{57}Fe	$\pm 2\sigma$	[Fe]
			Museum	Chemistry	(‰)		(‰)		(‰)		(μg/g)	(‰)		(μg/g)	(‰)		(‰)		(μg/g)
Aepyceros melampus		KNP	TM17677	L8[a]	1.16		1.74		2.05										
Aepyceros melampus		KNP	TM17679	L9[a]	1.08		1.54		1.98										
Aepyceros melampus		WC	TM 13260	L10[a]	0.60		0.97		1.14										
Philamtomba monticola	Blue duiker	WC	TM3061	Zn3[c]	0.96		1.27		1.87										
Philamtomba monticola	Blue duiker	WC	TM3059	Zn4[c]	1.01		1.61		2.03										
				Zn4[c]	1.00		1.56		1.98										
				38Zn[b]	0.93		1.22		1.68										
					0.98	*0.09*	*1.46*	*0.42*	*1.90*	*0.37*	82								
Philamtomba monticola	Blue duiker	WC	TM3146	Zn5[c]	0.75		1.19		1.31										
Raphicerus melanotis	Cape grysbok	WC	TM9333	40Zn[b]	0.55		0.92		1.07		335	−0.10							
												−0.07							
												−0.09	*0.04*	297*					
Tragelaphus scriptus	Bushbuck	WC	TM3220	Zn6[c]	0.69		1.29		1.22										
				Zn6[c]	0.67		1.17		1.38										
				33Zn[b]	0.70		1.13		1.35										
					0.68	*0.03*	*1.20*	*0.17*	*1.31*	*0.17*		−0.17		3	−0.12		−0.18		276
Plant residues																			
Taurotragus oryx	Common eland	KNP	TM16994PR	L31[a]	0.07		0.07		−0.04										
				L15[a]	0.14		0.35		0.42										
					0.11	*0.10*	*0.21*	*0.40*	*0.19*	*0.64*									
Redunca arundinum	Southern reedbuck	KNP	TM16659PR	L33[a]	−0.03		−0.09		−0.09										
				L32[a]	−0.04		0.01		−0.13										
				L29[a]	−0.09		−0.24		−0.16										
				46Zn[b]	0.06		0.15		0.12										
					−0.03	*0.12*	−0.04	*0.34*	−0.06	*0.26*	116				0.19		0.29		2080
Equus burchelli	Zebra	KNP	TM16690PR	L21[a]	0.35		0.46		0.62										
				47Zn[b]	0.47		0.84		1.10			−0.78							
				47Zn[b]	0.52		1.03		1.21			−0.94							
					0.45	*0.18*	*0.78*	*0.58*	*0.97*	*0.63*	43	*−0.86*	*0.23*	12	0.11		0.09		5665
Hippotragus niger	Sable antelope	KNP	TM16999PR	44Zn[b]	0.41		0.48		0.75										
				44Zn[b]	0.42		0.62		0.71						−0.08		−0.13		3565
					0.42	*0.01*	*0.55*	*0.19*	*0.73*	*0.05*	64								
Sylvicapra gimmia	Common duiker	KNP	TM16661PR	L26[a]	0.25		0.64		0.64										
Aepyceros melampus	Impala	KNP	TM17679PR	Zn17[c]	0.27		0.41		0.51										
Sylvicapra grimmia	Common duiker	KNP	TM4081PR	Zn7[c]	0.28		0.46		0.48			−0.78		1.6	0.15		0.17		1334
Equus burchelli	Zebra	KNP	AZ1132PR	L22[a]	0.33		0.41		0.66										
Giraffa camelopardalis	Giraffe	KNP	TM12254PR	L14[a]	0.21		0.42		0.58										
Tragelaphus scriptus	Bushbuck	WC	TM3220PR	32Zn[b]	0.05		0.00		0.11			−0.11		8	0.07		0.22		5141
Aepyceros melampus	Impala	WC	TM13260PR	34Zn[b]	0.25		0.33		0.63										

[a] Method *M*2 and P54 measurement.
[b] Method *M*1 and Nu500 measurement.
[c] Method *M*2 and Nu500 measurement.

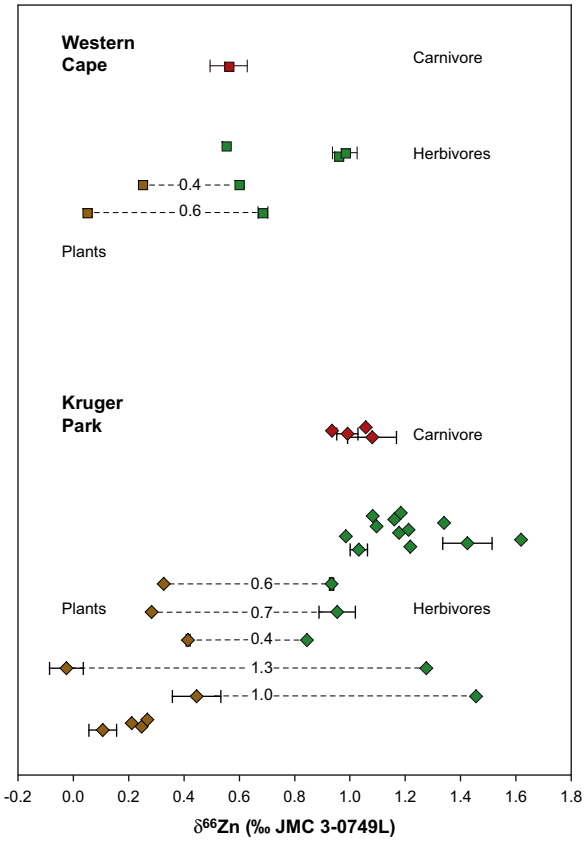

Fig. 3. Zn isotopic values for carnivores, herbivores and plants in KNP and WC. Brown symbols stand for plants, green for herbivores and red for carnivores. A dashed line links teeth and associated plant residues. The corresponding Zn isotope fractionation value ($\Delta^{66}Zn_{p-b}$) is given. Errors bars are the 2σ deviation of replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

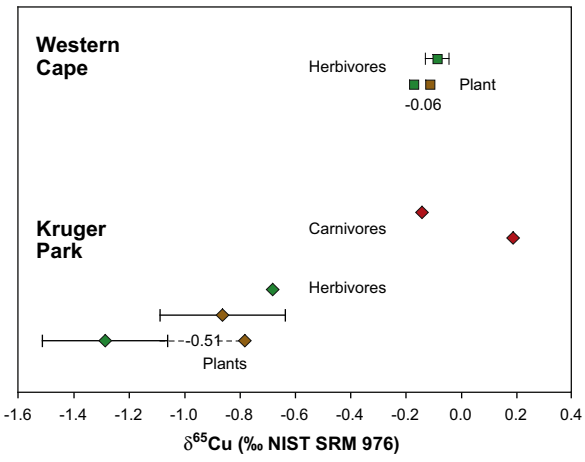

Fig. 4. Cu isotopic values for carnivores, herbivores and plants in KNP and WC. Brown symbols stand for plants, green for herbivores and red for carnivores. A dashed line links teeth and associated plant residues. The corresponding Cu isotope fractionation value ($\Delta^{65}Cu_{p-b}$) is given. Errors bars are the 2σ deviation of replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the patterning of the Cu isotope compositions in plants and animals is very different from that of Zn. The Cu isotopic compositions of plants at KNP range from −0.94‰ to −0.78‰. These are distinct from the single plant measured at WC, which yields a δ^{65}Cu value of −0.11‰. Herbivores are characterized by δ^{65}Cu values ranging from −1.20‰ to −0.68‰ at KNP and from −0.17‰ to

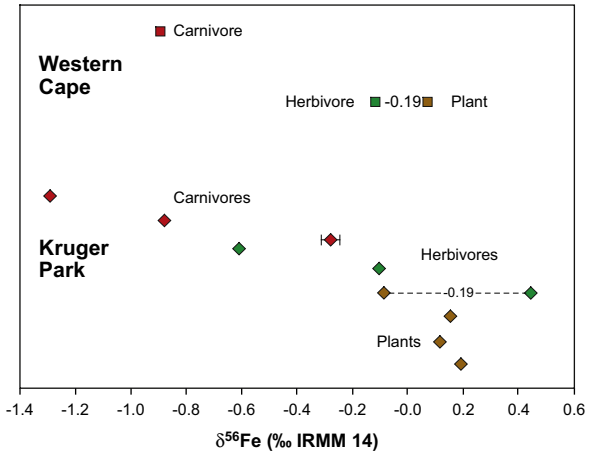

Fig. 5. Fe isotopic values for carnivores, herbivores and plants in KNP and WC. Brown symbols stand for plants, green for herbivores and red for carnivores. A dashed line links teeth and associated plant residues. The corresponding Fe isotope fractionation value ($\Delta^{56}Fe_{p-b}$) is given. Errors bars are the 2σ deviation of replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

−0.07‰ at WC. The two carnivores of KNP have δ^{65}Cu values of −0.14‰ and 0.19‰. There is no apparent Cu isotopic fractionation between plants and herbivores, but carnivores are highly ^{65}Cu-enriched relative to herbivores (Fig. 4).

3.3. Iron

The Fe concentrations range from 1.6 to 311 μg/g in bones and from 2080 to 5665 μg/g in plants. The typical range of Cu concentrations in mammal bones is 100–2000 μg/g (Jaouen et al., 2012). Iron concentrations in plants are extremely variable depending on the Fe uptake strategy but the range, to our knowledge, does not exceed few hundreds of μg/g (Oertli and Jacobson, 1960). Fe isotopic compositions range from −0.08‰ to 0.19‰ at KNP and are not different from a single δ^{56}Fe value of 0.07‰ measured at WC. Herbivores are characterized by δ^{56}Fe values ranging from −0.61‰ to 0.44‰ at KNP, encompassing the single δ^{56}Fe value (−0.12‰) reported for WC. However, carnivores are ^{56}Fe-depleted relative to herbivores: at KNP, the δ^{56}Fe of carnivores ranges from −1.29‰ to −0.25‰, and the only carnivore measured at WC has a δ^{56}Fe value of −0.89‰. The δ^{56}Fe values of herbivores are therefore not fractionated relative to plants, while carnivores are (Fig. 5).

3.4. Concentration outliers

Some metal concentrations values in bones and plants can be considered as outliers. We do not have definitive explanations but some hypotheses can be put forward. For bones, the concentration values that are below the lower limit of typical ranges (TM4404: [Zn] = 8 μg/g and [Fe] = 1.6 μg/g; TM4081: [Cu] = 0.93 μg/g) are the most difficult to explain. Leaching process of the carcass prior to the collection or bacterial alteration are possible processes. One Cu concentration value (TM9333, 297 μg/g) is higher than the typical range, and can be explained by the partial sampling of cancellous bone. For plants, the high Fe concentrations can be explained by the contamination of soil particles trapped with plant residue. All the plant residues analyzed were from grazers, which are animals that clip vegetation at or near ground level. Unfortunately, we did not have access to plant residues from browsers (e.g. giraffe), which are animals that feed with woody twigs and leaves, i.e. vegetation that is not in contact with soil. The Fe concentrations

and hence isotopic composition of plants are probably contaminated by soils values.

3.5. Possible regional metal isotopic signatures

A Cu, and a possible Zn, isotopic offset exist between the KNP and WC for a given trophic step (Figs. 3 and 4). The plants and herbivore's bones at WC are ^{65}Cu-enriched by about 0.7‰ relative to those at KNP (Fig. 4). Unfortunately, as no bone of carnivore was measured at WC, it is not possible to test whether the carnivores at WC are also shifted by 0.7‰ compared to those at KNP. The herbivores at WC are ^{66}Zn-depleted compared to the herbivores at KNP by about 0.3‰, and the sole carnivore at WC is ^{66}Zn-depleted by about 0.5‰ relative to those at KNP (Fig. 3). However, plants have identical Zn isotope compositions in both trophic chains. Contrary to Cu and possibly Zn, there is no Fe isotopic offset between KNP and WC for a given trophic step (Fig. 5). In the absence of soils metal isotope values at KNP and WC, the interpretation of the above results remains speculative. However, the types of plants at KNP and WC are very different, with possible distinct metal uptake intensities and associated isotopic fractionations. At KNP, there is a very long dry season resulting in a savanna landscape composed of grassland with scattered shrubs and isolated acacia trees. The climate is under oceanic influences at WC and is characterized by the fynbos biome, which comprises evergreen heathlands with fine-leafed low shrubs and typical leafless tufted grasslike plants.

Table 3
Summarized isotopic fractionations values between trophic steps (Δ). The subscript c–h denotes the trophic fractionation between carnivores and herbivores bones, and the subscript h–p denotes the trophic fractionation between herbivores bones and plants. For each metal, $\Delta_{c-h}=\delta_c-\delta_h$ and $\Delta_{h-p}=\delta_h-\delta_p$.

	Area	δ^{66}Zn (‰)	$\pm 2\sigma$	n	$\Delta^{66}Zn_{c-h}$ (‰)	$\pm 2\sigma$	$\Delta^{66}Zn_{h-p}$ (‰)	$\pm 2\sigma$
Carnivores	KNP	1.03	0.16	11	−0.08	0.43		
Herbivores	KNP	1.11	0.40	30			0.89	0.55
Plants	KNP	0.23	0.38	16				
Carnivores	WC	0.52	0.05	2	−0.26	0.35		
Herbivores	WC	0.79	0.35	10			0.64	0.45
Plants	WC	0.15	0.28	2				
	Area	δ^{65}Cu (‰)	$\pm 2\sigma$	n	$\Delta^{65}Cu_{c-h}$ (‰)	$\pm 2\sigma$	$\Delta^{65}Cu_{h-p}$ (‰)	$\pm 2\sigma$
Carnivores	KNP	0.02	0.47	2	1.16	0.78		
Herbivores	KNP	−1.13	0.62	3			−0.29	0.64
Plants	KNP	−0.84	0.15	3				
Carnivores	WC			0				
Herbivores	WC	−0.11	0.09	3			0.01	0.09
Plants	WC	−0.11		1				
	Area	δ^{56}Fe (‰)	$\pm 2\sigma$	n	$\Delta^{56}Fe_{c-h}$ (‰)	$\pm 2\sigma$	$\Delta^{56}Fe_{h-p}$ (‰)	$\pm 2\sigma$
Carnivores	KNP	−0.60	0.92	4	−0.51	1.40		
Herbivores	KNP	−0.09	1.05	3			−0.18	1.08
Plants	KNP	0.09	0.24	4				
Carnivores	WC	−0.89		1	−0.77			
Herbivores	WC	−0.12		1			−0.19	
Plants	WC	0.07		1				

4. Discussion

4.1. Bodily isotopic fractionation and trophic patterning

One of the most striking features of the results is the general consistency of the Zn, Cu and Fe isotopic distributions between the KNP and WC (Figs. 3–5), despite the small number of samples analyzed at WC. In both trophic chains, the bones of herbivores do not seem to be fractionated in Cu and Fe relative to plants, while they are ^{66}Zn-enriched, relative to plants. Also, in both trophic chains, the bones of carnivores are ^{65}Cu-enriched, ^{56}Fe-depleted, and slightly ^{66}Zn-depleted, relative to bones of herbivores. The trophic enrichments are summarized in Table 3.

The distribution of the metal isotope compositions in the trophic chains can be the result of isotopic fractionation processes that occur at the level of the organism. Data coming from experiments on mammals fed a controlled diet can shed light on the origin of the isotopic trophic patterning (Balter et al., 2010; Hotz et al., 2011). These are summarized in Fig. 6. Bone and muscle have similar δ^{66}Zn values that are ^{66}Zn-enriched by about 0.2–0.3‰ relative to diet (Balter et al., 2010). The environmental data presented here show that the ^{66}Zn-enrichment between the bone of an herbivore and the plants it consumed ($\Delta^{66}Zn_{p-b}$) exhibits values ranging from 0.4‰ to 1.3‰ with a mean $\Delta^{66}Zn_{p-b}$ value of about 0.8 ± 0.4‰ (Fig. 3). The origin of the difference between the natural and experimental $\Delta^{66}Zn_{p-b}$ values is so far unknown, but can probably be attributed to intra-plant Zn isotopic variability, which can exceed 1.0‰ (Weiss et al., 2005; Moynier et al., 2009). As the plants sampled in between the teeth represent only what the animal ate the days before it died, the plants δ^{66}Zn values area partial, and most probably heterogeneous, snapshot of the overall diet. The trophic ^{66}Zn-enrichment observed between plants and bones of herbivores does not exist, surprisingly, between bones of herbivores and bones of carnivores. One mechanism can explain this discrepancy. It involves a reduced bioavailability of Zn in the presence of inhibitory substances found in plant products, such as phytates (Lönnerdal, 2000). The partial association of dietary Zn to phytates into the intestines might be associated with isotope fractionation processes during plant digestion by herbivores. Following a kinetic fractionation process, the incomplete binding of Zn to phytates should favor light Zn isotopes. Feces of sheep are ^{66}Zn-depleted relative to diet by about 0.2‰ (Balter et al., 2010), which is compatible with a preferential binding of light Zn isotopes to phytates. This fractionation processes is not relevant for carnivores because of the absence of phytate in animal products. Carnivores would absorb quantitatively Zn and therefore resemble herbivores. Balanced isotopic fractionation during Zn absorption and Zn release in the gastro-intestinal tract of carnivores, however, cannot be ruled out.

Experimental data on sheep and mice indicate that bone δ^{65}Cu values are consistently not fractionated relative to diet, while those of muscles are ^{65}Cu-depleted relative to diet (Fig. 6). A trophic ^{65}Cu-depletion is predicted to occur between bones of herbivores and bones of carnivores, but not between plants and bones of herbivores. Strikingly, the natural data suggest the opposite, in other words, that bones of carnivores are ^{65}Cu-enriched, not ^{65}Cu-depleted, relative to bones of herbivores and plants. The similarity of the δ^{65}Cu values of plants and bones of herbivores argues for a quantitative absorption of Cu by herbivores, which is in contrast with the absorption of Zn. While Zn-phytate complexes precipitate at pH characteristic of the gastrointestinal tract, phytates complexes involving Cu do not (Champagne and Fisher, 1990; Lönnerdal, 1996). This can explain the lack of Cu isotopic fractionation during plants digestion. The origin of the ^{65}Cu-enrichment between herbivores and carnivores remains unsolved and requires further experimental investigations.

The progressive ^{56}Fe-depletion up trophic chain is expected since the whole body of mammal is ^{56}Fe-depleted relative the diet (Walczyk and von Blanckenburg, 2002; Hotz et al., 2011). The Fe isotopic fractionation is likely to occur in the digestive tract, during the reduction of Fe^{3+} to Fe^{2+} by Fe-reductase (Andrews, 2005). The reduction of Fe, whether organic or inorganic, is accompanied

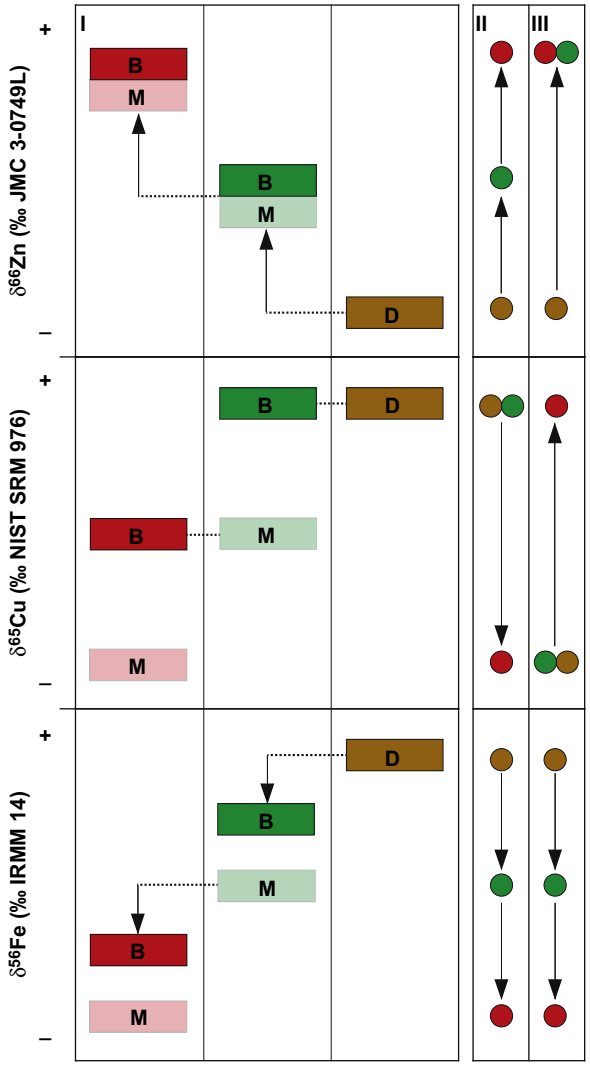

Fig. 6. Panel I: schematic distribution of the δ^{66}Zn, δ^{65}Cu and δ^{56}Fe values in experimental animals (Zn data are from Balter et al. (2010); Cu and Fe isotopic values are from Balter and Zazzo (2011): sheep fed with a diet with a δ^{65}Cu value of about 0.0‰ have bone at ~0.0‰ and muscle at ~−0.5‰; mice fed with a diet with a δ^{65}Cu value of about 0.3‰ have bone at ~0.3‰ and muscle at ~−0.2‰), between diet (D), muscle (M) and bone (B). Muscle of herbivores has been assumed to be the dietary source for carnivores. Panel II: expected trophic Zn, Cu and Fe isotopic fractionations from bodily isotopic distributions. Panel III: observed trophic Zn, Cu and Fe isotopic fractionations.

by a depletion of the ^{56}Fe/^{54}Fe ratio (Anbar et al., 2000). Because muscle is ^{56}Fe-depleted relative to bone, the bones of carnivores are ^{56}Fe-depleted compared to that of herbivores. The trophic distributions of the δ^{56}Fe values are therefore well predicted by experimental data (Fig. 6).

4.2. A call for the study of metal isotope compositions in apatite along with C and N in collagen

Collagen C and N isotope compositions are routinely measured in archeology for paleodietary and paleoecological purposes. The rationale is based on the existence of a more or less constant fractionation of C and N isotope compositions between the diet and tissues of animals, allowing to predict the C and N isotope composition of an animal's diet given the values measured in its tissues. Theoretically, three food sources can be partitioned using the C and N isotopic ratios. The observations can be matched for simple trophic relationships in laboratory and mesocosm experiments but it rarely exists in natural contexts because the diet of an animal is most often composed of more than three isotopically distinct sources. As only C and N isotopes are measured routinely, this leads in cases of omnivorous dietary behavior to underdetermined systems that are impossible to resolve. Particularly true is the case of ancient human populations, being hunter/gatherers or farmer/pastoralists, whose diet was complex (Lee-Thorp and Sponheimer, 2006). The measurements of the Fe, Cu and Zn isotopic ratios along with those of C and N will give more constraints for reconstructing diets resulting from the mixing of multiple food sources. The measurement of the C and N isotope compositions in bone collagen involves the extraction of approximately 5 mg of collagen, which means that more than 200 mg of bone needs to be dissolved for a comfortable measurement of the C and N isotopic ratios. After collagen extraction, the remaining mineral phase formed by apatite is discarded. This is regrettable because the wasted apatite material could be used for the study of the metal isotopic ratios. However, the collagen extraction protocol includes a demineralization step that uses HCl 1 M (Tuross et al., 1988), resulting in the mixing of dissolved apatite and diagenetic phases, mainly calcite and oxyhydroxides that contain diagenetic metals (Kohn et al., 1999). A step should be envisaged to purify with a dilute acid the fossil apatite from exogenous phases prior to sample demineralization. The amount of apatite saved by this technique would be at least twice to four times more than necessary for the measurements of the metal isotope compositions, which is approximately 50 mg.

5. Conclusions

We report data for Zn, Cu and Fe stable isotope compositions in plants, bones of herbivores and carnivores from two trophic chains located in the Western Cape area and in the Kruger National Park in South Africa. The first trophic step (between plants and bones of herbivores) is characterized by a ^{66}Zn-enrichement of about 0.8‰ whereas no significant isotopic fractionation is observed for Fe and Cu. The second trophic step (between bones of herbivores and bones of carnivores) is characterized by a ^{56}Fe-depletion of about 0.6‰, a ^{65}Cu-enrichment of about 1.0‰, and no significant Zn isotopic fractionation. The trophic patterns of the metal isotope compositions can be explained by bodily variations and differential intestinal metal absorption between herbivores and carnivores. The data already suggests that the Zn, Cu and Fe stable isotope compositions are potentially new paleodietary and paleoecological proxies, but further studies will be necessary to better understand their sources of variations, both at the level of the organism and of the trophic chain.

Acknowledgments

Many thanks are due to Teresa Kearney and the Ditsong National Museum of Natural History (formerly Transvaal Museum, Northern Flagship Institution), for facilitating access to the samples. We appreciate the comments of three anonymous reviewers that greatly improved the clarity of the paper.

References

Anbar, A.D., Roe, J.E., Barling, J., Nealson, K.H., 2000. Nonbiological fractionation of iron isotopes. Science 288, 126–128.
Andrews, N.C., 2005. Understanding heme transport. N. Engl. J. Med. 353, 2508–2509.
Albarède, F., Télouk, P., Lamboux, A., Jaouen, K., Balter, V., 2011. Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways. Metallomics 3, 926–933.
Balter, V., Zazzo, A., Moloney, A., Moynier, F., Schmidt, O., Monahan, F., Albarède, F., 2010. Bodily variability of zinc natural isotope abundances in sheep. Rapid Commun. Mass Spectrom. 24, 605–612.

Balter, V., Zazzo, A., 2011. An animal model (sheep) for Fe, Cu, and Zn isotopes cycling in the body. Mineral. Mag. 75, 476.
Balsberg Påhlsson, A.M., 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water Air Soil Pollut. 47, 287–319.
Bigeleisen, J., Goeppert-Mayer, M., 1947. Calculation of equilibrium constants for isotopic exchange reactions. J. Chem. Phys. 15, 261–267.
Burkhead, J.L., Gogolin Reynolds, K.A., Abdel-Ghany, S.E., Cohu, C.M., Pilon, M., 2009. Copper homeostasis. New Phytol. 182, 799–816.
Champagne, E.T., Fisher, M.S., 1990. Binding differences of Zn(II) and Cu(II) ions with phytate. J. Inorg. Biochem. 38, 217–223.
Guelke, M., von Blanckenburg, F., 2007. Fractionation of stable iron isotopes in higher plants. Environ. Sci. Technol. 41, 1896–1901.
Hotz, K., Augsburger, H., Walczyk, T., 2011. Isotopic signatures of iron in body tissues as a potential biomarker for iron metabolism. J. Anal. At. Spectrom. 26, 1347–1353.
Jaouen, K., Balter, V., Herrsher, E., Lamboux, A., Telouk, P., Albarède, F., 2012. Fe and Cu stable isotopes in archeological human bones and their relationship to sex. Am. J. Phys. Anthropol. 148, 334–340.
Johnson, C.M., Beard, B.L., Beukes, N.J., Klein, C., O'Leary, J.M., 2003. Ancient geochemical cycling in the Earth as inferred from Fe isotope studies of banded iron formations from the Transvaal Craton. Contrib. Mineral. Petrol. 144, 523–547.
Jouvin, D., et al., 2012. Stable isotopes of Cu and Zn in higher plants: evidence for Cu reduction at the root surface and two conceptual models for isotopic fractionation processes. Environ. Sci. Technol. 46, 2652–2660.
Kohn, M.J., Schoeninger, M.J., Barker, W.W., 1999. Altered states: effects of diagenesis on fossil tooth chemistry. Geochim. Cosmochim. Acta 63, 2737–2747.
Larson, P.B., Maher, K., Ramos, F.C., Chang, Z., Gaspar, M., Meinert, L.D., 2003. Copper isotope ratios in magmatic and hydrothermal ore-forming environments. Chem. Geol. 201, 337–350.
Lee-Thorp, J., Sponheimer, M., 2006. Contributions of biogeochemistry to understanding hominin dietary ecology. Yearb. Phys. Anthropol. 49, 131–148.
Lönnerdal, B., 1996. Bioavailability of copper. Am. J. Clin. Nutr. 63, 821S–829S.
Lönnerdal, B., 2000. Dietary factors influencing zinc absorption. J. Nutr. 130, 1378S–1383S.
Maréchal, C., Télouk, P., Albarède, F., 1999. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chem. Geol. 156, 251–273.
Maréchal, C., Albarède, F., 2002. Ion-exchange fractionation of copper and zinc isotopes. Geochim. Cosmochim. Acta 66, 1499–1509.
Markl, G., Lahaye, Y., Schwinn, G., 2006. Copper isotopes as monitors of redox processes in hydrothermal mineralization. Geochim. Cosmochim. Acta 70, 4215–4228.
Moynier, F., Pichat, S., Pons, M.L., Balter, V., Fike, D., Albarède, F., 2009. Isotopic fractionation and transport mechanisms of Zn in plants. Chem. Geol. 267, 125–130.
Oertli, J.J., Jacobson, L., 1960. Some quantitative considerations in iron nutrition of higher plants. Plant Physiol. 35, 683–688.
Pons, M.-L., Quitté, G., Fujii, T., Rosing, M.T., Reynard, B., Moynier, F., Douchet, C., Albarède, F., 2011. Early Archean serpentine mud volcanoes at Isua, Greenland, as a niche for early life. Proc. Natl. Acad. Sci. USA 108, 17639–17643.
Tuross, N., Fogel, M.L., Hare, P.E., 1988. Variability in the preservation of the isotopic composition of collagen from fossil bone. Geochim. Cosmochim. Acta 42, 929–935.
von Blanckenburg, F., von Wirén, N., Guelke, M., Weiss, D.J., Bullen, T.D., 2009. Fractionation of metal stable isotopes by higher plants. Elements 5, 375–380.
Walczyk, T., von Blanckenburg, F., 2002. Natural iron isotope variations in human blood. Science 295, 2065–2067.
Walczyk, T., von Blanckenburg, F., 2005. Deciphering the iron isotope message of the human body. Int. J. Mass Spectrom. 242, 117–134.
Weinstein, C., Moynier, F., Wang, K., Paniello, R., Foriel, J., Catalano, J., Pichat, S., 2011. Isotopic fractionation of Cu in plants. Chem. Geol. 286, 266–271.
Weiss, D.J., Mason, T.F.D., Zhao, F.J., Kirk, G.J.D., Coles, B.J., Horstwood, M.S.A., 2005. Isotopic discrimination of zinc in higher plants. New Phytol. 165, 703–710.
Zhu, X.K., O'Nions, R.K., Guo, Y., Reynolds, B.C., 2000. Secular variation of iron isotopes in north Atlantic Deep Water. Science 287, 2000–2002.