Fe and Cu Stable Isotopes in Archeological Human Bones and Their Relationship to Sex

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ABSTRACT Accurate sex assignment of ancient human remains usually relies on the availability of coxal bones or well-preserved DNA. Iron (Fe) and copper (Cu) stable isotope compositions ($^{56}Fe/^{54}Fe$ and $^{65}Cu/^{63}Cu$, respectively) were recently measured in modern human blood, and an unexpected result was the discovery of a ^{56}Fe-depletion and a ^{65}Cu-enrichment in men's blood compared to women's blood. Bones, being pervasively irrigated by blood, are expected to retain the $^{56}Fe/^{54}Fe$ and $^{65}Cu/^{63}Cu$ signature of blood, which in turn is useful for determining the sex of ancient bones. Here, we report the $^{56}Fe/^{54}Fe$, $^{65}Cu/^{63}Cu$, and $^{66}Zn/^{64}Zn$ ratios from a suite of well-preserved phalanxes ($n = 43$) belonging to individuals buried in the 17th and 18th centuries at the necropolis of Saint-Laurent de Grenoble, France, and for which the sex was independently estimated from pelvic bone morphology. The metals were purified from the bone matrix by liquid chromatography on ion exchange resin and the isotope compositions were measured by multiple-collector inductively coupled plasma mass spectrometry. The results show that, as expected from literature data on blood, male bone iron is depleted in ^{56}Fe and enriched in ^{65}Cu relative to female. No sex difference is found in the $^{66}Zn/^{64}Zn$ ratios of bone. The concentration and isotopic data show no evidence of soil contamination. Four samples of five (77%) can be assigned their correct sex, a result comparable to sex assignment using Fe and Cu isotopes in blood (81%). Isotopic analysis of metals may therefore represent a valid method of sex assignment applicable to incomplete human remains. Am J Phys Anthropol 148:334–340, 2012.

Sex assignment of human remains is a crucial step of forensic and paleoanthropological studies. Presently, sex determination most often relies on pelvic morphology (Bruzek, 2002; Murail et al., 2005) or ancient DNA analysis (Stone et al., 1996). When pelvic bones are lacking or deteriorated or when ancient DNA is not preserved, the sex assignment of human remains is uncertain.

Walczyk and von Blanckenburg, (2002) showed that the iron (Fe) stable isotope abundances (as represented by the $^{56}Fe/^{54}Fe$ ratio) of male and female blood were different, though with significant overlap. The sex factor indicated by Fe isotopes was confirmed by subsequent work (Krayenbuehl et al., 2005; Stenberg et al., 2005; Albarède et al., 2011) and also found for copper isotopes ($^{65}Cu/^{63}Cu$), but not for zinc isotopes, $^{66}Zn/^{64}Zn$ (Albarède et al., 2011).

Iron, copper, and zinc are three metals essential to human metabolism. Iron is present in hemoglobin for the transport of oxygen and is involved in electron transfer. Copper also plays a role in electron transfer in critical metabolic pathways, and the metabolic relationships between Cu and Fe are multiple. Zinc is present in more than 300 metalloproteins, most of which have enzymatic or structural properties (Cousins, 1985). Being essential elements, the concentrations of Fe, Cu, and Zn are regulated by the organism to be between the deficiency and toxicity levels.

For a given element, the abundances of stable isotopes in various biological compartments vary because bond energy changes with the mass of each isotope. The molecular configuration, redox conditions, and kinetics are known to be the main driving forces for isotopic variations. As illustrated by recent literature (Walczyk and von Blanckenburg, 2002; Krayenbuehl et al., 2005; Albarède et al., 2011), isotopic variability of essential elements potentially yields information on metabolic pathways and health conditions that may not affect metal concentrations outside of the natural variability. Iron has four stable isotopes: ^{54}Fe (5.8 %), ^{56}Fe (91.7 %), ^{57}Fe (2.2 %), and ^{58}Fe (0.28 %). Although studies of the variations of Fe stable isotope ratios are still at a very early stage, they have been documented for some human organs such as liver, hair, muscle, feces, urine, and nails (Ohno et al., 2004; Krayenbuehl et al., 2005; Stenberg et al., 2005; Walczyk and von Blanckenburg, 2005; Albarède et al., 2011). Zinc has five stable isotopes, ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, and ^{70}Zn, with respective average natural

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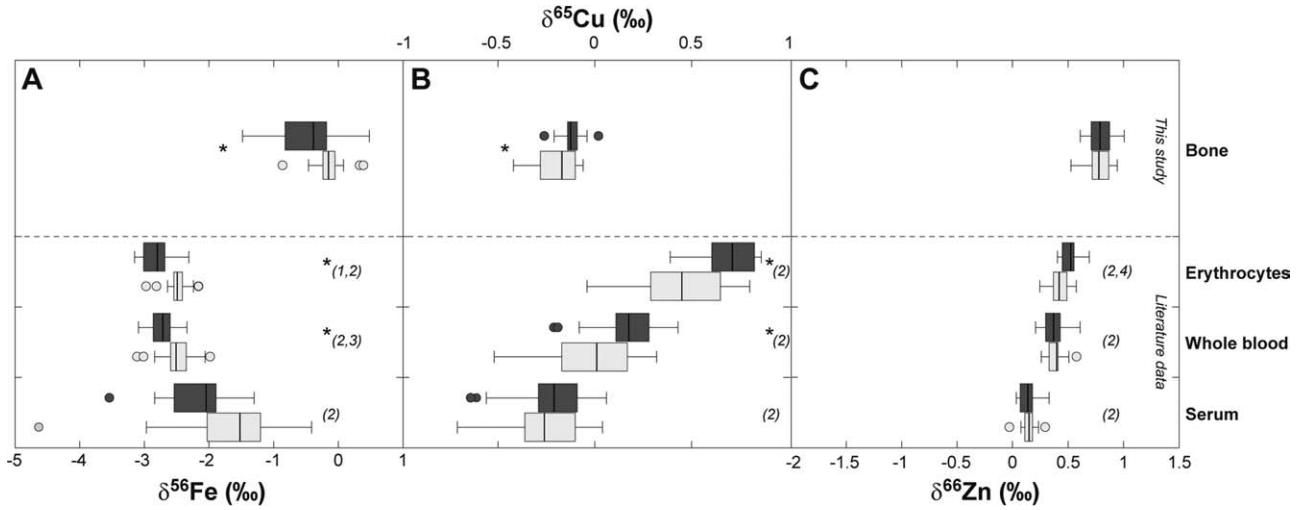

Fig. 1. Fe, Cu and Zn isotope variations of the organs discussed in the text. The delta annotations are given by: $\delta^x Fe = [(^xFe/^{54}Fe)_{sample}/(^xFe/^{54}Fe)_{standard}\text{-}1].10^3$ with $x = 56$ or 57). The "IRMM14" solution stands for the Fe isotope standard. $\delta^{65}Cu = [(^{65}Cu/^{63}Cu)_{sample}/(^{65}Cu/^{63}Cu)_{standard}\text{-}1].10^3$. The "NIST-SRM 976" solution was used as Cu isotope standard. $\delta^x Zn = [(^xZn/^{64}Zn)_{sample}/(^xZn/^{64}Zn)_{standard}\text{-}1].10^3$ with $x = 66$, 67, 68, or 70). The "JMC 3-0749" solution is the well-distributed Lyon Zn isotope standard. The box represents the 25th–75th percentiles (with the median as a bold vertical line) and the whiskers show the 10th–90th percentiles. Numbers in brackets correspond to references. Bone data are from this study. Statistically significant differences between women and men ($p < 0.02$) are indicated by an asterisk. Literature data: Walczyk and von Blanckenburg, 2002.; Albarède et al, 2011; Ohno et al, 2004; Ohno et al, 2005.

abundances of 48.6, 27.9, 4.1, 18.8, and 0.6%. Copper has only two stable isotopes, ^{65}Cu (30.8%) and ^{63}Cu (69.2%). While a first systematic investigation was published for the organs and body fluids of sheep by Balter et al. (2010), Zn and Cu stable isotope compositions of blood and hair constitute the only reference for humans (Ohno et al., 2005; Stenberg et al., 2005; Albarède et al., 2011). Blood Fe and Cu isotope compositions show that Fe is enriched in ^{56}Fe and Cu depleted in ^{65}Cu in women's blood relative to the values observed for men, yet with some overlap for Fe (Walczyk and von Blanckenburg, 2002, 2005; Albarède et al., 2011) (Fig. 1). For Fe, isotopic differences have been attributed to differential intestinal absorption between men and women (Walczyk and von Blanckenburg, 2002; Krayenbuehl et al., 2005; Walczyk and von Blanckenburg, 2005).

Bones are permanently irrigated by blood and participate in metal storage. Iron and copper in bones could, therefore, be recording the $^{56}Fe/^{54}Fe$ and $^{65}Cu/^{63}Cu$ ratios of blood. As bones can be fossilized, archeological implications are potentially important because fossil bones may preserve sex differences in their Fe and Cu stable isotope compositions. A vast literature already exists on the variability of Fe, Cu, and Zn concentrations in recent or archeological bones (Brätter et al., 1977; Lyengar et al., 1978; Lambert et al., 1982; Beattie and Avenell, 1992; Vuorinen et al., 1996; D'Haese et al., 1999; Martínez-García et al., 2005). While this variability may be indicative of ancient disease or postmortem contaminations, it does not seem to relate to sex. The range of their stable isotope compositions also remains undocumented. We, therefore, measured the Fe, Cu, and Zn stable isotope compositions in well-preserved bones from a suite of individuals recovered from 17th and 18th centuries French graves and for which the sex had previously been established from the morphology of the pelvic bones.

Stable isotope compositions of metals in bones may first be controlled by diet. Metal stable isotope compositions are known to vary among dietary foodstuffs: for instance, the Fe in animal products is depleted in ^{56}Fe relative to plant foodstuffs, presumably due to preferential intestinal absorption of light Fe isotopes (Walczyk and von Blanckenburg, 2005). Likewise, Zn stable isotope compositions differ between animal and vegetal products as a result of isotope fractionation during intestinal uptake, with ^{66}Zn being preferentially absorbed relative to ^{64}Zn (Balter et al., 2010). For copper, no such pattern is known, either for concentrations or for isotopic ratios (Byrne and Parris, 1987). The relative importance of plants versus animal consumption and the presence of C3 versus C4 plants in the diet can be assessed using the carbon and nitrogen stable isotope compositions ($^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively) of collagen (Herrscher, 2003; Balter et al., 2005; Herrscher and Le Bras-Goude, 2010). To separate the effects of sex and diet, we tested potential relationships between $^{13}C/^{12}C$ and $^{15}N/^{14}N$ and metal stable isotope compositions.

The original metal stable isotope compositions of bones can be affected by diagenesis. Iron forms colloidal phases that can be transported by groundwater and can entrain large amounts of copper and zinc. Secondary mineral phases, such as iron hydroxides, can enter the porosity of bones and overprint the original isotopic signatures. There is no mineralogical criterion for unambiguous detection of diagenesis (Pucéat et al., 2004). Here, we use combined measurements of concentrations and stable isotope compositions to evaluate whether the initial biological metal inventory has been perturbed by a diagenetic end-member. For any given element, addition of variable proportions of a soil component to the initial biogenic inventory is expected to produce a mixing line, i.e., a correlation between concentrations and isotopic compositions (Albarède, 1996). It will further be tested whether manganese, which has been used as a proxy of Fe diagenesis due to its association with Fe in Fe-Mn oxy-hydroxides (Kohn et al., 1999), can be correlated with the indicators of diagenetic mixing.

MATERIAL

Human skeletal material has been excavated from the cemetery of the Saint-Laurent de Grenoble Church, located on the right bank of the Isère river within Grenoble (Northern Alps, France; Colardelle, 2008). According to ^{14}C dates, 1,500 skeletons discovered in primary burials belong to the period from the early Middle Ages (5th century AD) to the 18th century AD (Colardelle, 2008). This particular archeological sample set was selected because the C and N isotope compositions of the skeletons, motivated by investigations of the diet and breastfeeding, are available (Herrscher et al., 2001; Herrscher, 2003). Furthermore, due to the good preservation of the skeletal material anthropological data such as sex and age at death has been collected for numerous individuals (Herrscher et al., 2006). For the present study, a subsample of metacarpals and/or phalanges of 20 females and 33 males from the 17th–18th century AD (Modern Period) were selected. Historical and archeological data indicated deterioration of the economical context and the social status of the inhabitants of the Saint-Laurent quarter during the Modern Period compared with the Medieval Period (Colardelle, 2008). Paleopathological data reveal a significant increase of infectious and dental lesions for both adults and subadults (Herrscher et al., 2006). The paleopathology of the sample also disclosed one case of syphilis (T39) and two cases of metabolic disorders with two individuals suffering from diffuse idiopathic skeletal hyperostosis (DISH, T02, T20) (Herrscher and Katzenberg, 2008).

Sex assessment was performed on the hip bone (os coxae) using morphoscopic (Bruzek, 2002) and morphometrical methods (Murail et al., 2005). Bruzek's (2002) method for determining sex relies on the combination of five characters that allows samples to be classified as female, male, or indeterminate, whereas Murail's (2005) method estimates the probability for an individual to be a female or a male. In our case, Bruzek's (2002) method was applied using the first three characters with a success of 91% and the five characters with a success of better than 95% (Bruzek, 2002: 165–166). For the Murail determination, the threshold retained for assigning sex was 95%. When the results were different for the two methods, no sex assessment was given. Assessment of age at death was done using two methods based on the modification of the auricular surface of the coxal bone (Lovejoy et al., 1985; Schmitt 2005). As for sex, the Lovejoy method attributes a range of age, whereas the Schmitt method assigns a probability within an age interval. The two "age at death" estimation methods do not always give the same range, requiring merged ranges to be considered. Individuals were divided into four categories of young (YA, less than 34 years old), mature (MA, 34–45 years old), old (OA, more than 45 years old), and unknown (U). This division allows comparison between groups with minimal bias despite uncertainties on the age at death. In addition, individuals with no fusion of the spheno-occipital-synchondrosis, the iliac crest, or the medial extremity of clavicle were assigned to the young age group.

ANALYTICAL METHODS

Sample preparation and chemical purification

Cortical bone samples were surface abraded and ground in an agate mortar. They were leached for 30 min with dilute acetic acid (0.1 N) to remove diagenetic mineral phases (mainly carbonates). Residual material was rinsed three times with distilled water, dried down, and dissolved in 4.5 N HNO_3. The dissolved samples were then evaporated to dryness and redissolved in 1 mL of 7 N HCl + 0.001% H_2O_2 and evaporated again to remove any traces of remaining nitric acid. Finally, samples were taken up in 1 mL 7 N HCl + 0.001% H_2O_2 and a 50-μL aliquot was taken for elemental concentration measurements. The remaining solution was processed for isotope analysis according to the technique of Maréchal and Albarède (2002) on a column filled with the AG MP-1 anion-exchange resin (100–200 mesh, Bio-Rad). Copper was eluted with 20 mL of 7 N HCl + H_2O_2 0.001%, iron with 10 mL of 2 N HCl + H_2O_2 0.001%, and zinc with 10 mL of 0.5 N HNO_3. The process was repeated for each element to further reduce the amount of matrix.

TABLE 1. Variability of the isotopic measurements for eight samples

Sample name	Mean (‰)	Range (‰) Min	Max	SD	*n*
$\delta^{65}Cu$					
CuO	−0.31	−0.36	−0.25	0.03	13
2L	−1.38	−1.46	−1.29	0.06	5
Cu49	−0.4	−0.49	−0.32	0.05	8
$\delta^{56}Fe$					
3H	−1.87	−2.1	−1.67	0.11	53
$\delta^{66}Zn$					
ZnO	0.64	0.57	0.67	0.04	8
40Zn	0.5	0.39	0.6	0.06	7
3L	−0.66	−0.72	−0.63	0.04	4
ZnST4	0.36	0.28	0.41	0.04	6

SD stands for the standard deviation of the mean, and *n* corresponds to the number of analyses.

Isotopic and elemental analyses

Copper and zinc stable isotope compositions were determined on a Nu-HR multiple-collector inductively coupled plasma mass spectrometer (MC-ICPMS) using wet plasma, while Fe was run on a large-radius high-resolution Nu-1700 MC-ICPMS using dry plasma and a resolution of 4,500. The samples were introduced by free aspiration in 0.05 N distilled HNO_3. For Cu and Zn, instrumental mass fractionation was corrected using elemental doping and standard-sample bracketing following the technique of Albarède et al. (2004). The reproducibility of Cu, Zn, and Fe isotopic ratios was 0.05 permil. Samples were randomized during analysis and duplicates were measured to check for systematic errors. The external reproducibility based on the repeated measurement of in-house standards is given in Table 1. Iron, copper, and zinc concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 CX).

Statistical analysis

Diagenesis is expected to increase the spread of metal concentrations. Data falling outside the two standard-deviation range about the mean were therefore considered outliers and eliminated (Indrayan, 2006). To validate the potential of Fe and Cu isotopes for sex assessment, we first tested the data using bilateral Student's *t*-tests (Table 3). We performed a MANOVA, a discrimi-

TABLE 2. Average isotope compositions in delta units (permil or ‰) and 95% range (2SD) for the isotope compositions of Zn, Cu, and Fe in the bones and p value for t test performed between male and female values

	δ^{56}Fe			δ^{65}Cu			δ^{66} Zn		
Bones	*N*	av	2SD	*n*	av	2SD	*n*	av	2SD
Women	16	−0.12	0.65	13	−0.20	0.25	13	0.77	0.25
Men	27	−0.45	0.85	18	−0.11	0.16	18	0.80	0.22
All	43	−0.33	0.82	31	−0.15	0.22	31	0.79	0.23
p		0.01			0.02			0.34	

Typical analytical uncertainties are 0.05‰.

nant analysis and a leave-one-out cross validation using PAST software (Hammer et al., 2001). Fisher test on δ^{56}Fe and δ^{65}Cu values was also used to look at differences between men and women. For statistical differences between age groups, the non-parametric Kruskal–Wallis test was used because of the number of samples per age group (Table 4). Age groups only include individuals for which age assignment is unambiguous (Supporting Information Appendix Table S1). Finally, Fisher's tests were used to assess the correlations between diet indicators and isotope ratios (Table 5).

RESULTS

Mass-dependent isotope fractionation

The isotope and concentration data are reported in Supporting Information Appendix Table S2 and a summary of the statistical results is given in Table 2. We checked that isotopic fractionation was mass-dependent for all the samples, i.e., that δ^{66}Zn/2 = δ^{67}Zn/3 = δ^{68}Zn/4, and δ^{57}Fe/3 = δ^{56}Fe/2 (Fig. 2). Different slope values would have indicated instrumental mass-independent fractionation.

Bone diagenesis

The two-sigma filter led to the exclusion of five data points (Supporting Information Table S2). Once these samples were excluded, the Fe, Cu, and Zn concentration ranges correspond to those observed in fresh bone ([Cu] = 1–50 ppm, [Zn] = 50–400 ppm, [Fe] = 100–2,000 ppm) (Lyengar et al., 1978; Martínez-García et al., 2005).

Concentrations and isotopic ratios are not significantly correlated regardless of the elements (Table 3). This observation suggests that diagenetic perturbation, either by addition of a diagenetic component or by selective leaching, was minimal and that isotope compositions of metals in fossil bones reflect the values in the living individuals.

Influence of diet and age

No correlation was found for either sex between δ^{13}C and δ^{15}N, on the one hand, and among δ^{56}Fe, δ^{65}Cu, and δ^{66}Zn, on the other hand (Table 5). The age at death and the metal stable isotope compositions also seem uncorrelated (Table 4). One exception should be noticed for younger men's values, which show lighter iron isotope compositions. This result could, however, be due to sampling bias: individuals who were younger than the old-age group (YA/MA, Supporting Information Table S1), show iron isotope values similar to the oldest ones.

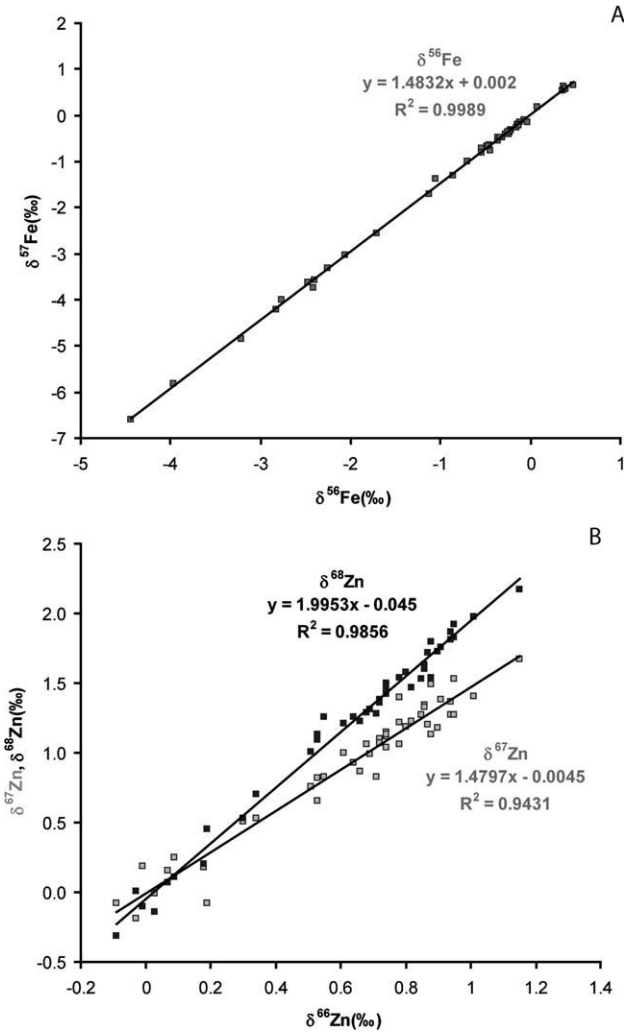

Fig. 2. Fractionation lines between different isotopes A: δ^{56}Fe vs. δ^{57}Fe; B: 1. δ^{67}Zn vs. δ^{66}Zn and δ^{68}Zn vs. δ^{66}Zn. All the samples fall on the theoretical mass-dependent fractionation lines.

TABLE 3. Correlation coefficients and associated p values between concentrations and isotopic ratio

	[Mn]		[Fe]		[Cu]		[Zn]	
	p	R^2	*p*	R^2	*p*	R^2	*p*	R^2
δ^{65}Cu	0.97	<0.001	0.67	0.0058	0.19	0.0491	0.22	0.0430
δ^{56}Fe	0.17	0.0412	0.79	0.0016	0.61	0.0059	0.93	0.0002
δ^{66}Zn	0.91	0.0003	0.58	0.0092	0.60	0.0081	0.49	0.0140

The sex factor

The average δ^{56}Fe, δ^{65}Cu, and δ^{66}Zn values in bones are −0.30 ± 0.38‰, −0.15 ± 0.11‰, and −0.79 ± 0.11‰, respectively. As for blood (Albarède et al., 2011), no significant sex difference is visible in the Zn stable isotope compositions of bones (Fig. 1). The values of women's bone δ^{56}Fe are higher by 0.33‰ than those of men (compared to 0.14‰ in blood). Women's bone δ^{65}Cu values are lower than men's values by 0.09‰ (compared to 0.16‰ in blood). The differences attributable to sex are statistically significant (bilateral *t* test, $p < 0.05$ for

TABLE 4. Chi-2 results for a Kruskal-Wallis test performed for Cu, Fe, and Zn isotope composition between pairs of age groups

	YA-MA			YA-OA			MA-OA			(YA+MA)-OA		
Age groups	δ^{65}Cu	δ^{56}Fe	δ^{66}Zn	δ^{65}Cu	δ^{56}Fe	δ^{66}Zn	δ^{65}Cu	δ^{56}Fe	δ^{66}Zn	δ^{65}Cu	δ^{56}Fe	δ^{66}Zn
All data	0.26	0.40	0.64	0.37	0.97	2.16	0.21	0.21	0.44	0.16	0.66	1.65
Women	0.00	0.02	0.86	0.86	0.15	0.86	0.00	0.00	2.40	0.44	0.07	0.00
Men	0.33	3.57	0.07	1.11	*4.72**	2.32	1.11	0.3	2.84	1.35	*3.94**	2.93

For two populations ($k - 1$) and a level of significance of 5% (± 0.05), χ^2 equals 3.84.OA = Old adults, >45 years old, MA = mature adults, 34–45 years old, YA= young adults, <34 years old.(YA+MA) corresponds to values for individuals classified in the age groups YA, MA and YA/MA.
Kruskal-Wallis tests are performed for both sexes taken together (All data) and separately (Women, Men).* is for significant results, i.e., when $p < 0.05$.

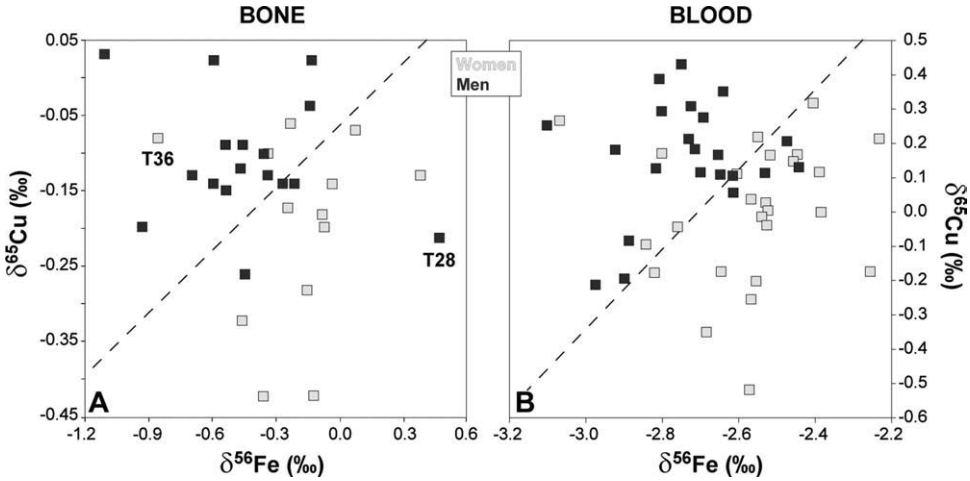

Fig. 3. Distribution of blood (B) and bone (A) δ^{56}Fe and δ^{65}Cu values for women and men. A discriminant analysis has been used for visually confirming or rejecting the hypothesis that male and female are isotopically distinct, which is represented by a discrimination line on the graphs. The percentage of correctly classified items is also given. A: Bone δ^{56}Fe and δ^{65}Cu discriminate women and men (Wilks' $\lambda = 0.74$; $F = 4.88$; $df = 2.28$; $p = 0.015$). The dashed line is the discriminant line between women and men and provides a correct sex classification in 24 cases of 31 (77%). B: Blood data come from Albarède et al. (2011). The assignment is correct for 81% of the samples.

TABLE 5. Correlation coefficients and associated p values between diet indicators and metal isotopes ratio

	δ^{65}Cu		δ^{56}Fe		δ^{66}Zn	
	R^2	p	R^2	p	R^2	p
δ^{13}C (‰)	0.101	0.08	0.017	0.41	0.018	0.47
δ^{15}N (‰)	0.002	0.78	0.004	0.68	0.036	0.31

both elements). The results for bones are illustrated in Figure 3A and those for blood (Albarède et al., 2011) in Figure 3B for comparison. In each panel, the line is defined by the best fit discrimination between men and women. The difference in δ^{56}Fe and δ^{65}Cu between the two groups is statistically significant (Fisher test, $F = 4.88$, $p = 0.02$). Sex assignment based on a leave-one-out cross validation with δ^{56}Fe and δ^{65}Cu values gives consistent results for bones and blood (77 and 81% of successful determinations for bones and whole blood, respectively).

DISCUSSION

Our results show that Fe and Cu stable isotope compositions in bones differ statistically between men and women, with Fe being enriched in ^{56}Fe and copper depleted in ^{65}Cu in women relative to men. The isotopic trend mirrors that of whole blood and erythrocytes (Walczyk and von Blanckenburg, 2002; Albarède et al., 2011) (Figs. 1 and 3). The overlap between the isotope compositions of Fe and Cu in male and female samples accounts for occasional errors when sex assignment is made based on the discrimination line. However, accuracy is comparable to that achieved for sex assessment based on isolated bones. For example, morphometric sex assessment for the metacarpals and/or phalanges we analyzed gives correct sex assignment for 74–94% of the samples (Scheuer and Elkington, 1993; Falsetti, 1995; Smith, 1996; Stojanowski, 1999; Case and Ross, 2007). In addition, morphometric data and resulting sex assignment depend on the population group studied (Case and Ross, 2007) and on the observers (Walrath et al., 2004). Although the extent of isotopic variability from one ethnic group to another still remains to be determined, the risk of interobserver bias is small.

Our data do not show Fe isotopic differences in bones between women who apparently died before the age of 45 and those who died after 45 (Table 5). The age of menopause, around the 50th year, has not varied much since medieval times (McKinlay et al., 1972; Amundsen and Diers, 1973; Thomas et al., 2001). This implies that either menstrual loss is not a major factor in defining the Fe isotope composition in blood of fertile women or Fe isotope compositions of bones take more than 10 years to reach

steady state with diet. The individuals affected by DISH and syphilis do not seem isotopically distinct from the others (Supporting Information Appendix Table S2).

Incorrect assignment of sex is observed for both bones and blood (Fig. 3). Bones of individuals T28 and T36 (Fig. 3A) clearly are outliers. Whereas investigation based on Bruzek's (2002) method suggest that the individual T36 was a female, Murail's (2005) method does not discriminate at a 95% confidence level ($p = 88$), which highlights the difficulty in obtaining a reliable sex attribution for this individual. In contrast, the sex assignment of the male T28 is unambiguous. A perturbation of the Fe and Cu status may account for the incorrect assignment of this individual. Indeed, clearly incorrect isotopic-based sex assignment is observed among the blood samples: i.e., female #15 (Fig. 3B) is characterized by iron depleted in ^{56}Fe and copper enriched in ^{65}Cu. Because all the samples from this dataset were collected from 20- to 35-year old healthy donors (Albarède et al., 2011), any effect from menopause can be disregarded. Individual T28, a male whose bone isotope compositions of Fe and Cu fall within the range of females' bones, may have suffered from hemochromatosis. This genetic disorder leads to hepatic Fe overload and is known to affect the Fe stable isotope composition of blood (Krayenbuehl et al., 2005; Stenberg et al., 2005), with patients' blood expectedly iron-enriched in ^{56}Fe relative to blood of healthy individuals. The cases of the blood of female #15 and the bone of female T36 remain more enigmatic. Validation of the present method would benefit from further studies of archeological series for which sex is unequivocally assigned through DNA sequencing or of recent series for which the identity of the deceased person is well established.

Metal stable isotope compositions are unlikely to be reliable paleodietary indicators because there is no apparent relationship between the measured isotopic abundances of metals, carbon, and nitrogen. In addition, preliminary data on total blood sampled from individuals with a vegetarian diet show no remarkable differences in Fe stable isotope compositions relative to omnivores (Walczyk and von Blanckenburg, 2005). During intestinal uptake, Fe and Zn isotopes are significantly fractionated, but fractionation at this stage seems to overwhelm the isotopic variability of different food sources (Walczyk and von Blanckenburg, 2005; Balter et al., 2010).

Finally, this study illustrates that metal isotope fractionation between bones and whole blood is similar for men and women. δ^{56}Fe and δ^{66}Zn are higher in bones relative to blood by about 2.3 and 0.39‰, respectively. Blood Cu is enriched in ^{65}Cu with respect to bones by 0.25‰. The cause of metal isotope fractionation between blood and bones is unclear. Metals must be supplied to osteoblasts for bone formation via the extracellular matrix in an exchangeable form. As a first approximation, serum is a good representative of extracellular fluids. The average copper isotope composition is thus similar in serum and bones (~ -0.11‰) (Albarède et al., 2011). Nevertheless, δ^{56}Fe (-1.25‰) and δ^{66}Zn (-0.62‰) in serum differ from δ^{56}Fe and δ^{66}Zn in bones, with apparent serum-bone fractionation of about -0.9‰ and $+1.4$‰, respectively (Fig. 1). Redox reactions involving Fe and Cu have been invoked as the origin of sex isotopic differences in blood (Albarède et al., 2011), but such reactions clearly do not account for Zn isotope variability. Substitution of the divalent cations Fe^{2+}, Cu^{2+}, and Zn^{2+} with Ca^{2+} in the bioapatite crystal lattice is well documented (Duff, 1975). In the extracellular matrix, Cu bound to ceruloplasmin (Kosman, 2002) is in its divalent form and therefore readily substitutes with Ca^{2+} with no or minor isotopic effect. Iron, in contrast, is bound to transferrin in the 3+ oxidation state and hence must first be reduced to Fe^{2+} before uptake by the bioapatite crystal lattice. Partial oxidation of Fe to the 3+ state at this stage may therefore account for the rather large iron isotopic differences between serum and bones while Cu remains essentially unchanged. The isotope fractionation of Zn between blood and bones, with bones being heavier than serum by 1.4‰, requires a different effect that can only be of configurational origin. This 1.4‰ difference is perfectly consistent with isotope fractionation at ambient temperature between Zn phosphates and Zn^{2+} predicted by *ab initio* calculations of Fujii et al. (personal communication).

CONCLUSIONS

This study has shown that bones record the blood isotopic pattern. Iron and copper stable isotopes differ statistically between men and women's bones, but the intrasex variability seems to be unrelated to age and diet. Zinc isotopes do not show a sex difference, which suggests a role of redox reactions in the isotope fractionation process. The Fe and Cu isotopic record in blood from bones of a recent archeological human series opens up new perspectives for sex assignment in paleoanthropological studies. However, further studies are necessary to strengthen the present results. First, the variability of metal stable isotope compositions in blood must be better documented in living populations in order to define the range of existing isotopic variations. Second, the method developed here should be validated on reference suites of human bones for which the age and the sex are known. This would be of interest for archeological human remains for which sex has been assigned using fossil DNA, or for historical human remains for which sex was recorded during autopsy. Third, experimental studies using animal models are potentially useful to the understanding of the isotopic fractionation between blood and bones.

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