JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

The Iron Stable Isotope Fingerprint of the Human Diet

Friedhelm von Blanckenburg,*[,†] Janine Noordmann,[§] and Monika Guelke-Stelling[#]

[†]Helmholtz Center Potsdam GFZ German Center for Geosciences Telegrafenberg, D-14473 Potsdam, Germany, and Institute of Geological Sciences, Freie Universität Berlin, Germany

[§]Institute of Mineralogy, Leibniz University of Hannover, Callinstrasse 3, D-30167 Hannover, Germany

[#]Karlsruhe Institute for Technology (KIT), Institute for Mineralogy and Geochemistry, Adenauerring 20b, D-76131 Karlsruhe, Germany

Supporting Information

ABSTRACT: The stable isotopes of iron disclose the metabolic pathways of iron within the human food chain. We have measured with precise multicollector ICP-MS the iron concentrations and stable isotope composition of 60 food products that are representative of the average German diet. We find that vegetables fall within the range typical of strategy I plants (−0.1 to −1.4‰ in δ^{56}Fe), crop products and processed crop foods into the range typical of strategy II plants (−0.6 to +0.4‰), and animal products into the ^{54}Fe-enriched range known for animal tissue and blood (−1.1 to −2.7‰). Weighting these isotope compositions by the average iron dietary sources, we find a representative composition of European vegetarian diet of −0.45‰, whereas that of omnivores is −0.82‰. For human blood, known to be enriched in light iron isotopes, we find fractionation factors for iron absorption of −2.0 and −2.3‰ for vegetarians (female and male, respectively) and −1.3 and −1.5‰ for omnivores (female and male, respectively). Knowing these fractionation factors is a prerequisite for using stable iron isotope ratios in blood as monitors of intestinal iron uptake regulation.

KEYWORDS: *human diet, metal stable isotopes, multicollector ICP mass spectrometry, biomedical*

INTRODUCTION

Iron stable isotopes now begin to serve as tracers of nutrient uptake paths, processes, and efficiencies in humans.[1] All humans contain a specific iron stable isotope signature in their blood. This novel isotope biomarker was disclosed with the introduction of precise multicollector ICP mass spectrometry.[2] Human blood and muscle tissue are enriched in the light iron isotope, ^{54}Fe, by one to two per mil (‰) over the heavy iron isotope, ^{56}Fe, when compared to initial surveys of the human diet.[1−4] As a model organism, the whole body of a minipig was found to be enriched by exactly 1.5‰ in ^{54}Fe over ^{56}Fe relative to its feed.[5] Yet the full exploitation of the potentially rich information contained in this biomarker is still impaired by our lack of an accurate description of the composition of the human diet. Knowing this composition is a prerequisite to (a) estimate the extent to which an individual's unique blood composition is affected by that individual's diet; (b) identify whether an individual's blood composition is solely due to fractionation during absorption or due to internal distribution between organs;[6,7] (c) identify whether the blood of patients affected by hemochromatosis approaches that of their diet as expected if the degree of uptake is high;[8] (d) single out whether ethnic differences in iron isotope composition, such as the reported difference between Swiss and Thai subjects,[9] are due to metabolic processes or due to differences in the diet; (e) attribute differences in the blood's iron isotope composition to uptake efficiency rather than to differences in the individuals diet;[9] and, finally, (f) explore whether a stable metal isotope fingerprint could serve as a tool for food authentication.[10]

Mapping out the iron stable isotope composition of the human diet is difficult as all forms of food differ widely in their composition: plant food of "strategy I" plant origin (most non-graminaceous plants) encompasses a range from 0 to −3‰ in ^{56}Fe/^{54}Fe, given that these plants use a reductive uptake strategy.[11−14] These plants also differ in the composition of their different parts, with fruits and highest leaves typically containing the strongest enrichment of the light iron isotope, ^{54}Fe.[11,13,14] In contrast, "strategy II" plants (graminaceous plants) feature a narrower range (from −0.5 to +0.2‰) in ^{56}Fe/^{54}Fe and do not differ between their parts.[11,13,14] Initial surveys of animal food show that most meat sources, except liver, are enriched in the light iron isotope to a degree similar to human blood (from −2 to −2.7‰ in ^{56}Fe/^{54}Fe).[2,5,7] This similarity to blood is explained by the heme molecule containing most iron in both blood and muscle tissues.

In this study, we have measured the iron concentrations and stable iron isotope compositions of 60 food products, which we purchased in German supermarkets. These products are representative of the average German diet. To calculate the representative diet's composition, we have weighted these results by the Fe dietary sources described for the typical German "food basket".[15] These estimates of the dietary iron intake are similar to those of the United States.[16] Finally, we have calculated typical fractionation factors for intestinal iron absorption in humans.

MATERIALS AND METHODS

Food Sources. As our aim is to characterize the middle European diet, we have guided our food survey by the health survey of the German

Received: May 31, 2013
Revised: November 1, 2013
Accepted: November 4, 2013
Published: November 4, 2013

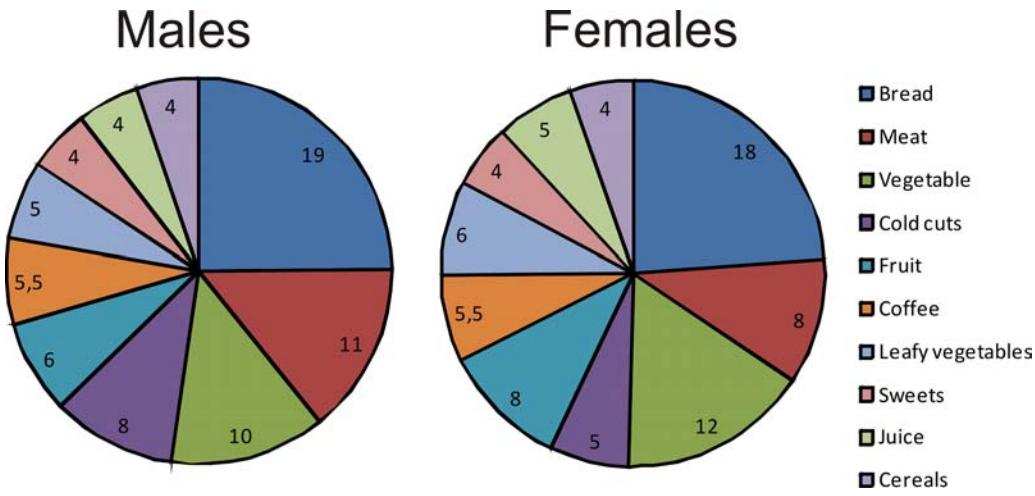

Figure 1. Components of the average German "food basket",[15] comprising 76.5% (males) and 75.5% (females) of the most important iron sources that are shown in percent.

Robert Koch Institute using the survey tool DISHES 98 (Dietary Interview Software for Health Examination Studies).[15] This report lists the eight most important Fe sources that account for 75% of Fe intake (Figure 1). The remaining 25% is distributed over a further 21 food sources. These are negligible as they either contain little iron or are consumed only rarely. The eight most important groups differ in their relative proportions between men and women (Figure 1). Forty-two food products were obtained in 2007 in a conventional German supermarket (Table 1). In addition, 18 products grown under certified organic "biologisch" agricultural conditions were obtained to ensure that our sampling was representative over all consumed food sources. For four plant foods multiple producers were measured to provide a first estimate of source-specific variability. It must be noted that iron stable isotope measurements were developed to explore the composition of geological materials,[17] mass spectrometric machine time is still precious, and the analysis of foodstuffs represents a formidable nonroutine analytical effort and cost that still prohibits the analysis of a larger population of samples. This study can be hence considered as an upper limit for such survey at the current state of the method's capacity.

Sample Preparation. All analytical work was undertaken at the Institute of Mineralogy at the Leibniz University of Hannover. All samples were dried down to accurately calculate the concentrations in the sample on the basis of their dry weight. Plant samples were dried at 80 °C for 2 days in an oven, ground to mince if necessary, and homogenized. As we did not check for the attainment of full dryness, the iron concentrations reported in Table 1 should be regarded as good approximates. As the samples' iron concentrations are not an aim of this survey, and isotope ratios do not depend on initial sample weights, this potential inaccuracy is immaterial to this study. Samples of animal origin were freeze-dried to −18 °C, and the pressure was reduced to 0.3 mbar to sublimate the water.

Samples were prepared for iron isotopic analysis following the method of Schoenberg and von Blanckenburg.[17] About 300 mg of each sample was decomposed by microwave agitation at 200 °C in 8 mL of concentrated nitric acid (15 M) and 1 mL of 30% hydrogen peroxide, evaporated on a hot plate in Teflon beakers, and treated with 600 μL of 30% H_2O_2 to oxidize any remaining organic compounds and ferrous iron to ferric iron. Solutions were clear after these steps and devoid of any solid residue. Due to the small amount of iron in some samples (<5 μg/g), 300 mg of weighted sample was not sufficient for precise measurements of iron isotopes by MC-ICP-MS. These samples were decomposed in multiple batches that were recombined for measurements. The concentrations of iron and the inorganic matrix were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Following elemental analysis the samples were redissolved in 6 M HCl to separate the iron from the inorganic matrix by anion-exchange chromatography (resin DOWEX AG 1X8, 100−200 mesh) with quantitative recovery, evaporated, and dissolved in 1 mL of 0.3 M HNO_3.[17] Previous studies revealed that Fe separates of samples with high transition metal contents or organic matrices may not be entirely matrix-free after anion-exchange chromatography and require further purification.[17] To ensure an interference-free measurement from the mass spectrometer, an additional precipitation step was applied to ensure a quantitative precipitation of all $Fe^{(III)}$ as $Fe^{(III)}OOH$, whereas Cu, Zn, Co, Cd, Mn, and V as well as organic compounds remain in solution. The samples were precipitated at pH ≈10 with 25% NH_3, and solutions were equilibrated for 1 h before centrifugation. The supernate solutions were discarded; the precipitates were washed twice with Milli-Q H_2O and then redissolved in HNO_3. The Fe concentration was measured before and after the precipitation step with ICP-OES, to ensure a near-quantitative (±10%) recovery during precipitation, which is essential to avoid Fe isotope fractionation. The samples were diluted to 3−6 μg/g Fe in 0.3 M HNO_3 for isotopic analysis.

Concentration Measurements. Quantitative recovery and removal of matrix elements during iron separation and precipitation were controlled on small aliquots of the dissolved samples before and after each step by ICP-OES (Varian Vista PRO CCD Simultaneous). After sample decomposition, the iron concentrations of all samples were obtained by ICP-OES on a dry weight basis (Table 1). We estimate the total analytical uncertainty of the concentration measurements, comprising the uncertainty of sample weight and the uncertainty of the ICP-OES measurement, to be around 5%. Total procedural blanks were also measured and were found to be between 12 and 27 ng, a negligible amount compared to the samples' Fe.

Iron Isotope Measurements. The determination of the ratios of the stable iron isotopes was carried out by a multiple collector inductively coupled plasma mass spectrometer (MC-ICP-MS; Neptune, Thermo Finnigan located at the Institute of Mineralogy at the Leibniz University of Hannover) according to the analytical protocol described by Schoenberg and von Blanckenburg.[17] The sample-standard bracketing technique was used to correct for instrumental mass bias using the Fe standard material IRMM-014, which was obtained from the Institute for Reference Materials and Measurements, Geel, Belgium, and has a certified $^{56}Fe/^{54}Fe$ isotope ratio of 15.69859. Iron isotope data are reported as $\delta^{56}Fe$ relative to the IRMM-014 standard, of which the isotopic composition is close to that of rocks at the Earth's surface[18,19] (defined as $\delta^{56}Fe = 0$), as defined in eq 1:

$$\frac{\delta^{56}Fe_{sample}}{‰} = \left(\frac{(^{56}Fe/^{54}Fe)_{sample}}{(^{56}Fe/^{54}Fe)_{IRMM\text{-}014}} - 1 \right) \times 1000 \quad (1)$$

To check for molecular or elemental interferences, all $\delta^{56}Fe$ and $\delta^{57}Fe$ of the samples were plotted against each other and were found to follow a mass-dependent fractionation law.

Table 1. Sources, Fe Concentrations, and Fe Isotope Composition of Measured Foodstuffs[a]

	concn Fe[b] (μg g^{-1})	δ^{56}Fe[c] (‰)	2σ[d] (‰)
Strategy I Plant Products			
arugula salad	201.0	−0.166	0.108
broccoli	44.8	−1.059	0.059
Brussels sprouts	38.0	−1.421	0.076
field salad	107	−0.396	0.004
field salad (organic)	345.7	−0.087	0.015
flax seed (organic)	35.4	−1.116	0.059
iceberg lettuce	65.3	−0.908	0.059
peas 1	35.7	−0.609	0.059
peas 2	52.2	−0.859	0.113
peas (organic)	92.1	−1.137	0.160
potato	8.1	−0.802	0.082
potato (organic)	10.2	−0.924	0.059
spinach 1	127	−0.434	0.059
spinach 2	85.6	−0.509	0.151
spinach	92.8	−0.499	0.062
spinach (organic)	83	−0.355	0.085
zuccini	45.4	−0.697	0.059
zuccini (organic)	47.1	−0.982	0.082
Strategy II Plant Products			
oats (organic)	31.7	0.133	0.059
oatmeal 1	55.9	0.294	0.059
oatmeal 2	55	0.253	0.075
oatmeal 3	56.4	0.393	0.059
oat flakes (organic)	36.8	0.257	0.059
wheat flour type 1050 1	21.3	−0.126	0.059
wheat flour type 1050 2	21.9	−0.251	0.059
whole wheat flour	40.2	−0.138	0.102
whole wheat flour	30.1	−0.124	0.092
whole wheat flour (organic)	23.5	−0.328	0.059
white rice	5.1	−1.189	0.059
brown rice	10.1	−0.942	0.190
Mushrooms			
brown	19.6	−0.076	0.112
white (organic)	15.5	−0.431	0.195
Animal Products			
beef	80.4	−1.975	0.059
egg yolk	147.8	−2.419	0.059
egg yolk (organic)	113.5	−2.114	0.096
egg white	0.8	−1.519	0.059
chicken breast	10.2	−2.555	0.059
chicken breast (organic)	8.6	−2.696	0.097
boiled ham	21.5	−2.083	0.059
boiled ham (organic)	17.4	−2.005	0.059
pork liver	623.5	−1.155	0.059
soft cheese	1.8	−1.416	0.059
minced meat sausage	13.8	−1.824	0.059
tuna	37.7	−0.722	0.140
salted herring	17.5	−2.466	0.059
Processed Food			
multigrain bread	25.1	−0.294	0.059
wheat bread (organic)	27.4	−0.134	0.059
rye bread (organic)	21.7	−0.410	0.059
wheat roll	10.7	−0.246	0.059
crispbread	28.8	−0.433	0.059
egg noodle	14.7	−0.481	0.059
egg noodle (organic)	15.7	−0.561	0.059
Drinks and Sweets			
coffee	23.1	−0.761	0.059
cocoa	316.6	0.091	0.100
green tea	417.4	0.127	0.059
Assam tea (organic)	100.1	0.123	0.071
English Breakfast Tea (organic)	123.6	0.133	0.074
black tea (organic)	78.4	0.130	0.073
Darjeeling tea (organic)	111.8	0.351	0.162
Roibos tea	73.3	−0.165	0.059

[a]The full data set is available in the Supporting Information. [b]Concentrations on a dry weight basis. [c]Average of replicate dissolutions was available. [d]2σ error of mass spectrometric measurement, of individual replicate dissolutions, or that calculated from 64 pooled replicate dissolutions (2σ δ^{56}Fe = 0.059‰), whichever is larger.

As an additional quality check of isotope ratio measurements, a commercially available pure Fe wire (99.998% purity, lot NM36883) from Johnson & Matthey (hereafter referred to as JM) has been measured throughout all mass spectrometric sessions. Sixty-six measurements of the internal JM standard gave the following results: δ^{56}Fe = 0.424 ± 0.065‰ (2σ); δ^{57}Fe = −0.610 ± 0.099‰ (2σ); δ^{58}Fe = 0.871 ± 0.434‰ (2σ). These values are identical to those reported by Schoenberg and von Blanckenburg.[17] Sixteen food samples were decomposed twice, to check for reproducibility and sample homogeneity. We achieved external reproducibilities of 0.059‰ for δ^{56}Fe and 0.107‰ for δ^{57}Fe. In Table 1, we report the uncertainty of iron stable isotope ratios as the reproducibility of repeat measurements or the external reproducibility of the 16 repeat decompositions, whichever was larger.

RESULTS AND DISCUSSION

Iron Concentrations and Iron Isotope Ratios. The analyzed food products yielded a spread in iron concentration from 0.8 to 624 μg/g and in δ^{56}Fe from −2.7 to 0.39‰ (Table 1). The analyzed strategy II plants yielded the highest δ^{56}Fe, between −0.56 and 0.39‰, and lower concentrations ranging from 5 to 56 μg/g. These ranges are similar to those found in growth experiments.[11,13] The analyzed strategy I plants contain mostly a lighter iron isotope composition than strategy II plants (from −1.4 to −0.1‰) and iron concentrations mostly between 38 and 200 μg/g (with two exceptions that are discussed below). Again, these values are within the range of growth experiments. The enrichment of ^{54}Fe in strategy I plants is due to the reduction during uptake,[20,21] which favors the light isotope (^{54}Fe) in the reduced compartment.[22,23]

Exceptions to these general rules are presented by rice and tea. Both white and brown rice, even though being strategy II plants, contain a light Fe isotope composition, with δ^{56}Fe of −0.9 and −1.2‰, respectively. This can be explained by the fact that rice, growing under anoxic conditions, does not suffer from Fe deficiency in waterlogged soils and is not required to invoke the $Fe^{(III)}$ complexing to combat deficiency otherwise characterizing well-aerated soils.[21,24] The case for tea, being a strategy I plant, yet featuring δ^{56}Fe from 0.1 to +0.4‰ and also unusually high concentrations of 120−350 μg/g, is more difficult to explain. One possibility is that tea leaves are contaminated during growth

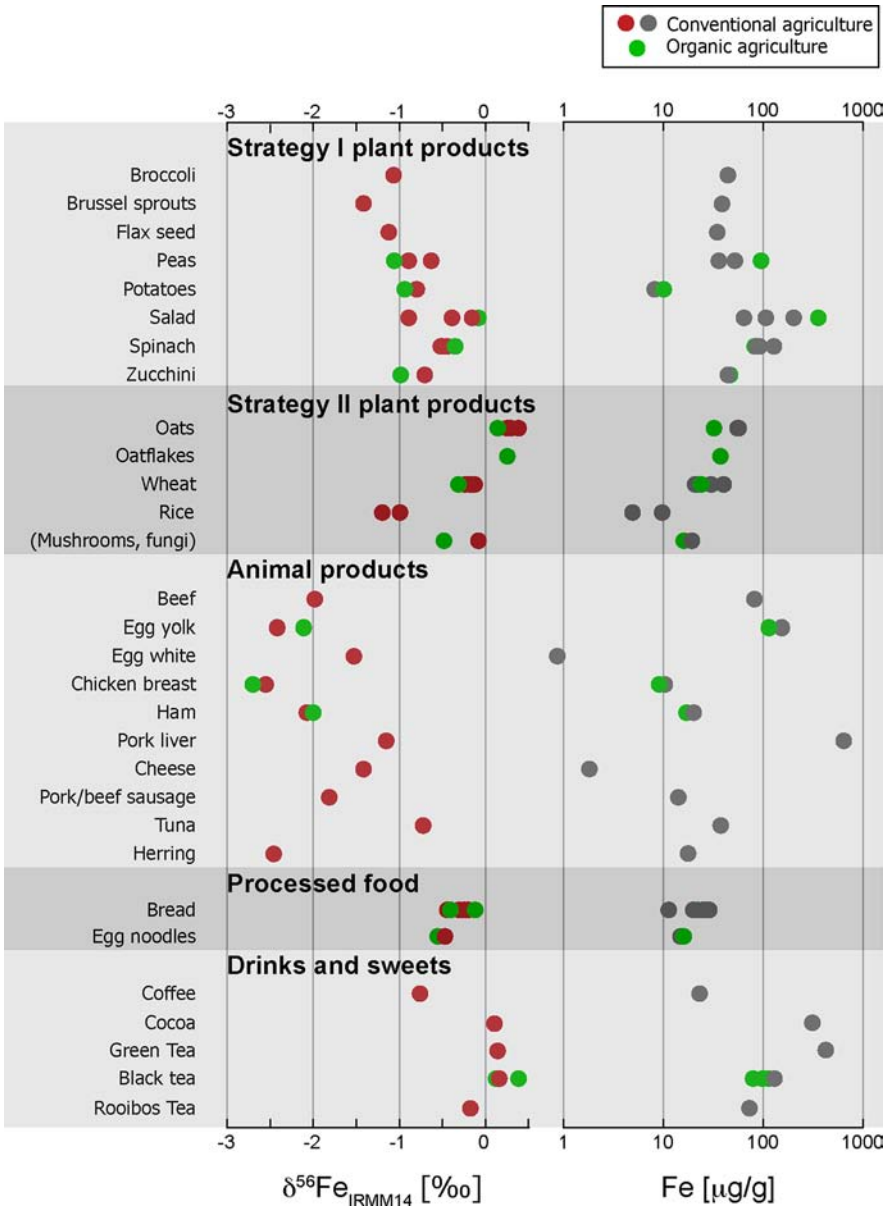

Figure 2. Iron isotope composition and iron concentrations (on a dry weight basis) of measured food products.

by atmospheric dust, containing iron with δ^{56}Fe of 0.1−0.2‰.[25] Another possibility is that all the processing of tea (fermenting, drying, sieving) provides frequent exposure to steel surfaces in the absence of washing during processing.

Processed food (noodles, bread, rolls) fall into the strategy II range, which is expected as their main Fe source is flour from crop plants. However, these products are on average slightly (~0.1‰) lighter in their iron isotope composition than strategy II plants.

Animal products yield the largest range in both δ^{56}Fe, ranging from −2.7 to −1.1%, and their iron concentration, ranging from 10 to 620 μg/g. However, egg white and soft cheese contain even less Fe, with 1−2 μg/g.

In general, meat products are enriched in the light iron isotope (^{54}Fe), as expected for muscle tissue that contains mostly heme-bound iron and hence a light Fe isotope composition.[1] With δ^{56}Fe of −1.1‰, pork liver contains the heaviest iron isotope composition and with 624 μg/g also the highest iron concentration of all analyzed animal products. Again, these values are expected as the liver is a store of ferritin-bound Fe that contains more ^{56}Fe than ^{54}Fe in blood.[5]

The two fish samples yield contrasting results: whereas herring contains Fe with δ^{56}Fe of −2.5‰, which is as isotopically light as iron in pork, beef, or chicken muscle, tuna contains Fe with δ^{56}Fe as heavy as −0.72‰. This comparatively heavy animal Fe isotope composition is found only in seafood and is compatible with the heavy Fe isotope composition found in tuna and shrimp muscle in the initial survey of Walczyk and von Blanckenburg.[2] This phenomenon might be related to the high myoglobin content of tuna muscle. This feature sets tuna apart from most other fish.

The analyzed chicken egg is interesting as Fe is fractionated between egg yolk (δ^{56}Fe of −2.4‰) and egg white (δ^{56}Fe of −1.5‰). The Fe concentrations are also vastly different, with only 1 μg/g for the egg white and 150 μg/g for the egg yolk.

In general, organically grown foods feature isotope ratios and concentrations similar to those of the counterparts sourced in conventional agriculture (Figure 2). Addition of extraneous iron to soil might potentially lead to deviations of δ^{56}Fe from the

common range in plants grown in conventional agriculture, as such additions potentially differ in their isotope composition from that of "plant-available" iron in soil.[10] Also, iron from average strategy II plants and processed food derived from strategy II plants cannot be explained by Fe fortification, as both the concentrations and isotope compositions are similar. These initial results might hint at stable iron isotopes possibly serving as tools for food authentication, but more food surveying is required to map out possible applications of this potential tool in detail.

Average Middle European Diet. When we average the found iron isotope ratios into the groups designated by the German "food basket",[15] we find the average values shown in Table 2 and Figure 3. These do not differ fundamentally from the average U.S. diet.[16] We mainly see two groups: animal products at δ^{56}Fe of around −2‰ and plant products from around −0.7 to 0‰. Hence, it is necessary to calculate the average diet separately for omnivorous and vegetarian individuals. Weighting the isotope compositions of Table 2 with the relative dietary Fe intake, we obtain a δ^{56}Fe of −0.77‰ for female omnivores and −0.46‰ for female vegetarians for their average diet. We obtain a δ^{56}Fe of −0.87‰ for male omnivores and −0.44‰ for male vegetarians as the composition of their average diet.

Table 2. Average Fe Isotope Composition and Their Standard Deviation of the Main Dietary Fe Sources

	δ^{56}Fe (‰)	σ (‰)	diet, females[a] (% Fe)	diet, males[a] (% Fe)
bread	−0.30	0.12	18	19
vegetable	−0.85	0.35	12	11
meat	−1.93	0.82	8	10
leafy vegetable	−0.42	0.25	6	6
coffee	−0.77		5.5	5.5
cold cuts	−1.97	0.13	5	4
sweets	0.09		4	4
corn, cereal	−0.21	0.48	4	4

[a]According to ref 15.

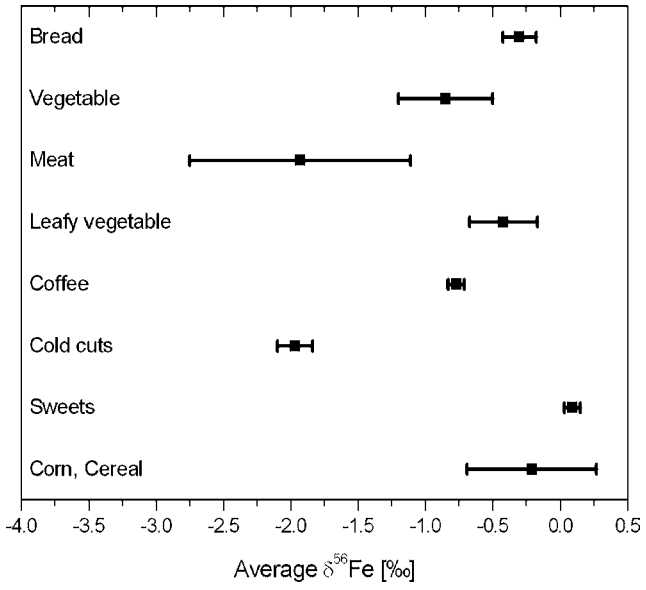

Figure 3. Average composition and standard deviation of the groups of diets using the relative amounts taken up and the isotope compositions from Table 2.

Isotope Fractionation during Intestinal Absorption. To calculate the average fractionation factor for intestinal absorption, we need to take into account that heme-bound iron is absorbed with an efficiency of 15−35% and nonheme iron absorption with 2−20%.[16] Nonheme iron absorption depends systematically on ferritin concentration in blood and varies from 14% at a ferritin concentration of 15 μgL^{-1} to 4% at a ferritin concentration of 60 μgL^{-1}.[26] For our calculation we use an efficiency of 30% for heme-bound iron and of ca. 10% for nonheme iron.[1] To take this effect into account, we weigh the mean diet isotope composition by these efficiencies and the iron consumption (Table 2). We used the average δ^{56}Fe of blood in young European adults that is based on three studies: Walczyk and von Blanckenburg[1,2] yielded an average δ^{56}Fe of −2.78 ± 0.19‰ for 21 males and −2.45 ± 0.19‰ for 31 females and Albaréde et al.[4] an average δ^{56}Fe of −2.74 ± 0.16‰ for 22 males and −2.57 ± 0.19‰ for 25 females. The average of all samples from these three studies is −2.76 ± 0.18‰ for males and −2.50 ± 0.20‰ for females, from which we obtained a fractionation factor for intestinal absorption of −1.3‰ for female omnivores and −2.0‰ for female vegetarians. We obtain a fractionation factor of −1.5‰ for male omnivores and −2.3‰ for male vegetarians, respectively.

That vegetarians and omnivores still do not differ in their bloods' composition can be explained by the fact that all heme-bound Fe passes through the intestinal mucosa without ligand exchange, thereby maintaining its light isotope composition, whereas all plant Fe, which is mostly present in the ferric form, is reduced in the intestine and is fractionated in the process to the heme Fe composition found in human blood. Hence, ultimately, all iron obtains a heme-like isotope composition.

Human Food Chain. It was shown by Walczyk and von Blanckenburg[1] that Fe is enriched as Fe is passed along the human food chain. This pattern was confirmed recently in a study of bones from herbivore and carnivore mammals.[27] Using our much more representative dietary data set in conjunction with soil data that explicitly resolved the plant-available soil fraction,[12,28−30] we can confirm this picture (Figure 4). We see that soils contain a mobile fraction of −0.05 ± 0.2‰, which is reflected in the average of all strategy II plants (0.07 ± 0.25‰),

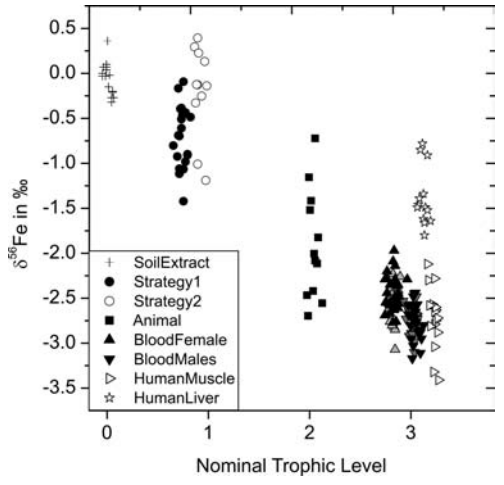

Figure 4. Iron isotope fractionation along the human food chain. Soil data represent the weakly bound (HCl-leachable) iron fraction.[12,28−30] All plant and animal data are from this study. Blood, muscle tissue, and liver data are from Walczyk and von Blanckenburg,[1,2] and blood data shown in gray symbols are from Albaréde et al.[4]

taking a minor fractionation toward ^{56}Fe by ligand exchange in the rhizosphere into account.[12] Adding strategy I plants yields an average vegetarian diet of −0.4‰ (see above). Animal food displays on average δ^{56}Fe of −1.9‰, whereas human blood and muscle tissue contain a δ^{56}Fe of roughly −2.6‰. Adding the liver as containing roughly 25% of human iron, we get an approximate composition for the human body of −2.5‰. The human body is therefore the lightest member of the human food chain.

Finally, we note that stable isotope ratio measurements of metal and metalloid elements that are now routine with multicollector ICP-MS open the possibility to explore metabolism along the food chain in a variety of elements. This high potential with the possibility to serve in certain aspects of food authentication is demonstrated by promising biomarker experiments on plants that have been made for metals, for example, for Mg,[31,32] Ca,[33] Cu,[7,34] Zn,[7,34,35] and Ni,[36] and for metalloids, for example, B[37] and Si.[38] First steps have been made in mapping out these metal biomarkers, and these new avenues now need to be explored in detail.

■ ASSOCIATED CONTENT

Supporting Information

Full dataset summarized in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(F.v.B.) E-mail: fvb@gfz-potsdam.de.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This research was supported by the German National Science Foundation DFG Grant BL562-6.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Ronny Schoenberg for support in mass spectrometry, Herdis Schopka for graphics, Thomas Walczyk for comments on a first version of the manuscript, and two anonymous reviewers and Nathalie Vigier for their constructive reviews.

■ REFERENCES

(1) Walczyk, T.; von Blanckenburg, F. Deciphering the iron isotope message of the human body. *Int. J. Mass Spectrom.* **2005**, *242*, 117−134.

(2) Walczyk, T.; von Blanckenburg, F. Natural iron isotope variations in human blood. *Science* **2002**, *295*, 2065−2066.

(3) Ohno, T.; Shinohara, A.; Kohge, I.; Chiba, M.; Hirata, T. Isotopic analysis of Fe in human red blood cells by multiple collector-ICP-mass spectrometry. *Anal. Sci.* **2004**, *20*, 617−621.

(4) Albarède, F.; Telouk, P.; Lamboux, A.; Jaouen, K.; Balter, V. Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways. *Metallomics* **2011**, *3*, 926−933.

(5) Hotz, K.; Augsburger, H.; Walczyk, T. Isotopic signatures of iron in body tissues as a potential biomarker for iron metabolism. *J. Anal. At. Spectrom.* **2011**, *26* (7), 1347−1353.

(6) Hotz, K.; Krayenbuehl, P. A.; Walczyk, T. Mobilization of storage iron is reflected in the iron isotopic composition of blood in humans. *J. Biol. Inorg. Chem.* **2012**, *17*, 301−309.

(7) Balter, V.; Lamboux, A.; Zazzo, A.; Télouk, P.; Levverrier, Y.; Marvel, J.; Moloney, A. P.; Monahan, F. J.; Schmidt, O.; Albarède, F. Contrasting Cu, Fe, Zn isotopic patterns in organs and body fluids of mice and sheep, with emphasis on cellular fractionation. *Metallomics* **2013**, DOI: 10.1039/C3MT00151B.

(8) Krayenbuehl, P. A.; Walczyk, T.; Schoenberg, R.; von Blanckenburg, F.; Schulthess, G. Hereditary hemochromatosis is reflected in the iron isotope composition of blood. *Blood* **2005**, *105*, 3812−3816.

(9) Hotz, K.; Walczyk, T. Natural iron isotopic composition of blood is an indicator of dietary iron absorption efficiency in humans. *J. Biol. Inorg. Chem.* **2013**, *18*, 1−7.

(10) Ashurst, P. R.; Dennis, M. J. *Analytical Methods of Food Authentication*; Blackie Academic: London, UK, 1998; p 350.

(11) Guelke, M.; von Blanckenburg, F. Fractionation of iron isotopes in higher plants. *Environ. Sci. Technol.* **2007**, *41*, 1896−1901.

(12) Guelke, M.; von Blanckenburg, F.; Staubwasser, M.; Schoenberg, R.; Stuetzel, H. Determining the stable iron isotope signature of plant-available iron in soils. *Chem. Geol.* **2010**, *277*, 269−280.

(13) Guelke-Stelling, M.; von Blanckenburg, F. Fe isotope fractionation caused by translocation of iron during growth of bean and oat as models of strategy I and II plants. *Plant Soil* **2012**, *352*, 217−231.

(14) Kiczka, M.; Wiederhold, J. G.; Kraemer, S. M.; Bourdon, B.; Kretzschmar, R. Iron isotope fractionation during Fe uptake and translocation in alpine plants. *Environ. Sci. Technol.* **2010**, *44*, 6144−6150.

(15) Mensink, G. *Beiträge zur Gesundheitsernährung des Bundes: Was essen wir heute? Ernährungsverhalten in Deutschland*; Robert Koch Institut: Berlin, Germany, 2002; p 168.

(16) Leong, W. I.; Loennerdal, B. Iron nutrition. In *Iron Physiology and Pathophysiology in Humans*; Anderson, G. J., MacLaren, G. D., Eds.; Humana Press: New York, 2012; pp 81−99.

(17) Schoenberg, R.; von Blanckenburg, F. An assessment of the accuracy of stable Fe isotope ratio measurements on samples with organic and inorganic matrices by high-resolution multicollector ICP-MS. *Int. J. Mass Spectrom.* **2005**, *242*, 257−272.

(18) Dauphas, N.; Pourmand, A.; Teng, F. Z. Routine isotopic analysis of iron by HR-MC-ICPMS: how precise and how accurate? *Chem. Geol.* **2009**, *267*, 175−184.

(19) Schoenberg, R.; von Blanckenburg, F. Modes and scales of intra-planetary iron isotope fractionation. *Earth Planet. Sci. Lett.* **2006**, *252*, 342−359.

(20) Kim, S. A.; Guerinot, M. L. Mining iron: Iron uptake and transport in plants. *FEBS Lett.* **2007**, *581*, 2273−2280.

(21) Kraemer, S. M.; Crowley, D. E.; Kretzschmar, R. Geochemical aspects of phytosiderophore-promoted iron acquisition by plants. *Adv. Agron.* **2006**, *91*, 1−46.

(22) Anbar, A. D.; Jarzecki, A. A.; Spiro, T. G. Theoretical investigation of iron isotope fractionation between $Fe(H_2O)_6^{3+}$ and $Fe(H_2O)_6^{2+}$: Implications for iron stable isotope geochemistry. *Geochim. Cosmochim. Acta* **2005**, *69*, 825−837.

(23) Welch, S. A.; Beard, B. L.; Johnson, C. M.; Braterman, P. S. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III). *Geochim. Cosmochim. Acta* **2003**, *67*, 4231−4250.

(24) Guerinot, M. L. It's elementary: enhancing Fe3+ reduction improves rice yields. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7311−7312.

(25) Beard, B. L.; Johnson, C. M. Fe isotope variations in modern and ancient Earth and other planetary bodies. In *Geochemistry of Non-Traditional Stable Isotopes*; Johnson, C. M., Beard, B. L., Albarède, F., Eds.; Mineralogical Society of America: Blacksburg, VA, 2004; Vol. 55, pp 319−357.

(26) Collings, R.; Harvey, L. J.; Hooper, L.; Hurst, R.; Brown, T. J.; Ansett, J.; King, M.; Fairweather-Tait, S. J. The absorption of iron from whole diets: a systemtic review. *Am. J. Clin. Nutr.* **2013**, *98*, 65−81.

(27) Jaouen, K.; Pons, M. L.; Balter, V. Iron, copper and zinc isotopic fractionation up mammal trophic chains. *Earth Planet. Sci. Lett.* **2013**, *374*, 164−172.

(28) Kiczka, M.; Wiederhold, J. G.; Frommer, J.; Voegelin, A.; Kraemer, S. M.; Bourdon, B.; Kretzschmar, R. Iron speciation and isotope fractionation during silicate weathering and soil formation in an alpine glacier forefield chronosequence. *Geochim. Cosmochim. Acta* **2011**, *75*, 5559−5573.
(29) Wiederhold, W. G.; Teutsch, N.; Kraemer, S. M.; Halliday, A. N.; Kretzschmar, R. Iron isotope fractionation during pedogenesis on redoximorphic soils. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1840−1850.
(30) Wiederhold, J. G.; Teutsch, N.; Kraemer, S. M.; Halliday, A. N.; Kretzschmar, R. Iron isotope fractionation in oxic soils by mineral weathering and podzolization. *Geochim. Cosmochim. Acta* **2007**, *71*, 5821−5833.
(31) Black, J. R.; Epstein, E.; Rains, W. D.; Yin, Q. Z.; Casey, W. H. Magnesium-isotope fractionation during plant growth. *Environ. Sci. Technol.* **2008**, *42* (21), 7831−7836.
(32) Bolou-Bi, E. B.; Poszwa, A.; Leyval, C.; Vigier, N. Experimental determination of magnesium isotope fractionation during higher plant growth. *Geochim. Cosmochim. Acta* **2010**, *74*, 2523−2537.
(33) Cobert, F.; Schmitt, A. D.; Bourgeade, P.; Labolle, F.; Badot, P. M.; Chabaux, F.; Stille, P. Experimental identification of Ca isotopic fractionations in higher plants. *Geochim. Cosmochim. Acta* **2011**, *75*, 5467−5482.
(34) Jouvin, D.; Weiss, D. J.; Mason, T. F. M.; Bravin, M. N.; Louvat, P.; Zhao, F.; Ferec, F.; Hinsinger, P.; Benedetti, M. F. 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.* **2012**, *46*, 2652−2660.
(35) Moynier, F.; Pichat, S.; Pons, M. L.; Fike, D.; Balter, V.; Albarède, F. Isotopic fractionation and transport mechanisms of Zn in plants. *Chem. Geol.* **2009**, *267*, 125−130.
(36) Cameron, V.; Vance, D.; Archer, C.; House, C. H. A biomarker based on the stable isotopes of nickel. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10944−10948.
(37) Rosner, M.; Pritzkow, W.; Vogl, J.; Voerkelius, S. Development and validation of a method to determine the boron isotopic composition of crop plants. *Anal. Chem.* **2011**, *83*, 2562−2568.
(38) Opfergelt, S.; Cardinal, D.; Henriet, C.; Dray, X.; Andre, L.; Delvaux, B. Silicon isotopic fractionation by banana (*Musa* spp.) grown in a continuous nutrient flow device. *Plant Soil* **2006**, *285*, 333−345.