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27. Other Gashatan and Bumbanian collections represented in Fig. 3 include Zhigden Member (ZG) and Members I (GS1), 2 (GS2), and 3 (GS3) of Khashat Formation, Ulan Nur Basin, Mongolia; Aguyt Member (AG), Naran Bulak Formation, Nemegt Basin, Mongolia; Bugin Member (BT) and Khaychin-Ula 1 fauna (KU), Bugin Tsav Basin, Mongolia; Nomogen Formation (NM), Erlian Basin, Inner Mongolia, China; Taizicun Formation (TZ), Dabu Formation (DB), and Shisanjianfang Formation (SS), Turfan Basin, Xinjiang Region, China; Pinghu Formation (PH), Chijiang Basin, Jiangxi, China; Shuangtasi Formation, Xuancheng Basin (ST), Shuangtasi Formation, Tongling Basin (TL), and Zhangshanji Formation (ZS), Laian Basin, Anhui, China; Yuhuangding Formation (YH), Henan, China; Ningjianshan Member (NJ) of Xinyu Group, Jiangxi, China; and Akasaki Formation (AK), Japan.
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Natural Iron Isotope Variations in Human Blood

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Isotopic analysis of human blood and liver and muscle tissue indicates that each individual bears a long-term iron (Fe) isotope signature in the blood. Blood and tissue differ slightly in isotopic composition and are depleted by up to 2.6 per mil in ^{56}Fe relative to ^{54}Fe when compared to dietary Fe. The ^{56}Fe/^{54}Fe isotope ratio in the blood of males is, on average, lower by 0.3 per mil than that of females. These results suggest that Fe isotope effects in the blood reflect differences in intestinal Fe absorption between individuals and genotypes.

Iron (Fe) is essential to the human body for oxygen transport in blood, for oxygen storage in muscle tissue, and as an enzyme cofactor. Iron deficiency anemia (IDA) affects 600 million to 700 million individuals worldwide, and women of childbearing age and children are most vulnerable (*1*). Fe deficiency develops when net Fe losses exceed Fe uptake. Within limits, this can be compensated for by the human body by liberating Fe from the stores in the liver and bone marrow for hemoglobin synthesis. When Fe stores are being depleted, intestinal Fe absorption is increased in parallel (*2*). When stores are empty and up-regulated Fe absorption fails to compensate for Fe losses, the hemoglobin concentration in the blood falls and IDA develops.

Conventional measures of Fe status allow the identification of Fe deficiency and IDA according to population-based reference values. However, because of strong day-to-day variations in Fe absorption, there is no suitable measure to identify whether Fe absorption is up-regulated in the individual to maintain Fe balance or, when Fe supply is abundant, down-regulated to avoid Fe overload.

Here we show that Fe isotope fractionation effects may serve as a tool to identify long-term differences in dietary Fe absorption between individuals. Iron isotope ratios have been commonly measured to study the absorption and utilization of dietary Fe in humans. Such studies use isotopically enriched Fe as tracers (*3*). Sophisticated mass-spectrometric techniques now allow the measurement of small changes in Fe isotope ratios produced by natural processes (*4*). The transport of matter alters the isotopic composition of an element if transfer between reservoirs is incomplete and if the transfer rate is related to the isotope masses. Variations in the ^{56}Fe/^{54}Fe isotope ratio of 1 to 2 per mil (‰) can be induced in natural samples both by microbial activity (*5*) and abiotic processes (*6*, *7*).

Using multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS), we assessed whether the human body discriminates between Fe isotopes and whether Fe isotope effects can be used to trace Fe transport processes in the body. Fe isotopes were analyzed in human blood samples and in liver and muscle tissue and excreta, as well as the main dietary Fe sources of plant and animal origin (*8*).

All blood samples had low Fe isotope ratios relative to the Fe standard of nonbiological origin (Fig. 1). Iron isotope ratios differed by as much as –3.1‰ in the ^{56}Fe/^{54}Fe isotope ratio (δ^{56}Fe) and as much as –4.7‰ in the ^{57}Fe/^{54}Fe isotope ratio (δ^{57}Fe). These are the strongest isotope effects for Fe yet observed in nature. The δ^{56}Fe range in silicate earth is about –0.5‰ to +0.5‰ (*9*), and Fe in bulk soils is relatively uniform at δ^{56}Fe = 0.2‰ (*10*). Model experiments involving Fe-dissimilating bacteria displayed maximum isotope shifts of δ^{56}Fe = –1.3‰ (*5*), and δ^{56}Fe values on the order of –1.6 to 1.0‰ have been found for certain Fe ores (*5*).

All observed isotope shifts are mass dependent (Fig. 1). Our precision (2 SD) of ±0.10‰ for the δ^{56}Fe measurements and ±0.12‰ for the δ^{57}Fe measurements allowed us to resolve differences between individuals. Men and women differed in the Fe isotope composition of their blood (Figs. 1 and 2). Men have a

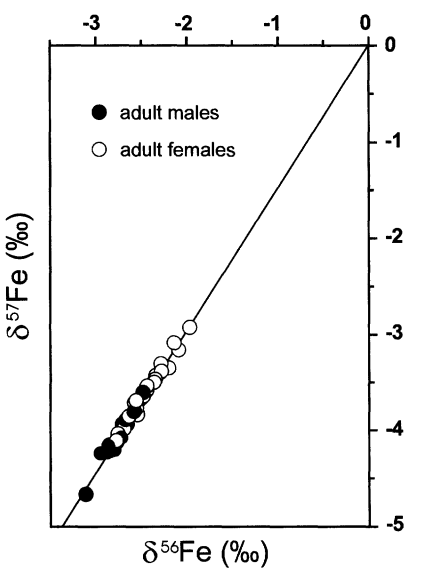

Fig. 1. Fe isotope variations in the blood of apparently healthy human adults. Each point represents an individual person. Data are plotted on a δ scale; that is, as the relative deviation in per mil from the reference isotope ratio (IRM-014, δ = 0). The diagonal line represents the predicted curve for a mass-dependent fractionation process according to an exponential law.

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greater proportion of lighter Fe isotopes in their blood than women (*11*). Four possible mechanisms may explain these data.

1) Fe in blood reflects dietary Fe, which is isotopically lighter than Fe in the geosphere. Fe from representative plant food sources was found to be slightly depleted in the heavier isotopes [δ^{56}Fe = –0.1 to –1.4‰ (Fig. 2)]. However, this depletion is insufficient to account for our blood data (δ^{56}Fe = –2.0 to –3.1‰). The same holds for seafood [shrimp and tuna muscle (Fig. 2)]. Fe isotope ratios in various animal products (beef, pork, chicken, and whole chicken eggs) are similar to those in human blood (Fig. 2); however, only 10 to 20% of dietary Fe intake comes from meat sources in a mixed diet (*12*). Because most Fe (>80%) leaves the body unabsorbed (*12*), the isotope composition of fecal Fe (δ^{56}Fe = about –1‰) was similar to that of dietary Fe (Fig. 2).

2) Fe isotopes are distributed heterogeneously within the human body, and Fe might be fractionated during distribution among various body reservoirs. Total body Fe averages ~3.8 g in men and ~2.3 g in women, which is distributed between red blood cells (~65%), liver (10 to 25%), and muscle/skin (~10%). The remainder is in enzymes, nails, and hair (<5%) (*12*). Our data indicate that Fe isotopes are distributed heterogeneously between blood (δ^{56}Fe range = –2.0 to –3.1‰), liver (δ^{56}Fe range = –0.9 to –1.6‰), and muscle tissue (δ^{56}Fe range = –2.1 to –3.4‰). Mass balancing of the isotopes taken in between the various reservoirs dictates that reservoirs complementary to the blood should have δ^{56}Fe values of +3 to +6‰. This has not been observed.

3) Iron losses from the body are biased in favor of the discharge of heavier Fe isotopes. Iron losses amount on average to about 1.0 mg/day in men and 1.3 mg/day in women through intestinal bleedings (~0.4 mg/day); bile (~0.2 mg/day); desquamated mucosal cells (~0.2 mg/day); sweat, skin, and hair (~0.3 mg/day); and urine (~0.3 mg/day). In addition, menstrual blood loss represents 10 to 15 mg of Fe per menstrual period (*12*). Only losses through bile, urine, tissue cells, and sweat can have an Fe isotope composition different from that of blood. Because Fe losses from the body are small in healthy adults (about 1 mg/day) (*12*), mass balancing requires that mean losses apart from those through blood have a δ^{56}Fe of +10‰. This hypothesis is not supported. Hair (in male adults) shows the highest depletion in the heavier isotopes: δ^{56}Fe of –3.3 to –3.6‰. Data from both urine and nail samples (*13*) further suggest that in these cases too there is no preferential loss of heavier Fe isotopes.

4) Light Fe isotopes are preferentially absorbed in the intestine. Fe(III) from plant foods is probably reduced to Fe(II) immediately at the mucosal surface before being bound to transport proteins that carry the Fe into the mucosal cell. The Fe is then partly released into the blood where it is taken up by transferrin as the Fe transport protein in plasma (*2*). Heme-bound Fe from meat products is transferred from the brush border to the intracellular environment, where the porphyrin ring is split to release Fe (*14*). A change in the Fe's redox state can be associated with fractionations in the parts per thousand range (*7*). Changes in the coordination of molecular bound Fe are also associated with an isotope fractionation effect (*6*, *15*–*17*). Finally, lighter Fe isotopes should be favored kinetically in transport processes (*18*).

The observed differences in the Fe isotope composition of blood between males and females are consistent with the hypothesis that the Fe isotope signature in blood is primarily determined by dietary Fe absorption. Average Fe losses and corresponding Fe requirements are higher in women. Higher Fe losses result in a poorer Fe status, to which the body responds by increasing Fe absorption (*19*).

Because only ~0.05% of the Fe inventory is replaced per day in adults (*12*), the Fe isotope composition of blood is not susceptible to short-term changes. Thus, the Fe isotope signature of blood could be used to study the long-term effect of genetic predisposition on dietary Fe absorption. Other applications may include studies on Fe transfer between the geosphere and the biosphere in the environmental sciences, tracing the origin of foods by their Fe isotope signature, and studies on food habits in anthropology and palaeontology based on the differences in Fe isotopic composition between foods.

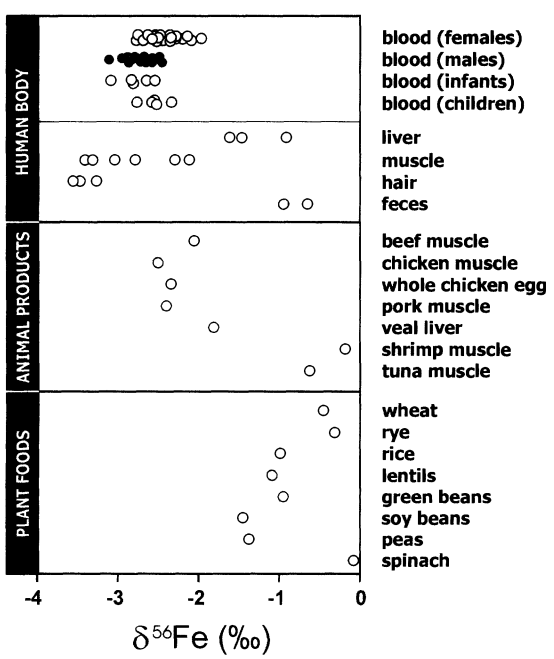

Fig. 2. Fe isotope variations in the human body and different food samples covering the most relevant dietary Fe sources. Data are plotted relative to an isotopic reference material (IRM-014, δ = 0).

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9. The average Fe isotope composition of terrestrial and lunar silicate rocks and Fe meteorites was determined at a δ^{56}Fe of ± 0.5‰ relative to Johnson Matthey iron (*20*). Repeated analyses of a Fe standard (Fe Purotronic wire, 99.998% purity, Johnson Matthey, lot NM36883) revealed differences in the Fe isotopic composition relative to certified iron isotope reference material (IRM-014) (δ^{56}Fe = 0.46 ± 0.10‰ and δ^{57}Fe = 0.68 ± 0.12‰, 2 SD, n = 50 independent runs).
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