Electronic Supplementary Information (ESI)

1.1 Derivation of equations used for calculation of NTBI amounts in serum samples (adapted from Walczyk et al, 1997)[1]

After adding ^{58}Fe spike into the filtered serum, the amount of natural iron present as NTBI in the mixture is n_{NTBI}, and the molar amount of ^{57}Fe spike and ^{58}Fe spike are $n_{Spk,57}$ and $n_{Spk,58}$, respectively. The molar amount of a certain iron isotope $n(^{m}Fe)$ with mass number m in the filtrate can be calculated from its natural isotopic abundance ($^{m}a_{NTBI}$), its isotopic abundance in the ^{57}Fe spike ($^{m}a_{spk,57}$) and its isotopic abundance in the ^{58}Fe spike ($^{m}a_{spk,58}$) as follows:

$$n(^{54}Fe) = n_{NTBI} \cdot {}^{54}a_{NTBI} + n_{spk,57} \cdot {}^{54}a_{spk,57} + n_{spk,58} \cdot {}^{54}a_{spk,58} \tag{S-1}$$

$$n(^{56}Fe) = n_{NTBI} \cdot {}^{56}a_{NTBI} + n_{spk,57} \cdot {}^{56}a_{spk,57} + n_{spk,58} \cdot {}^{56}a_{spk,58} \tag{S-2}$$

$$n(^{57}Fe) = n_{NTBI} \cdot {}^{57}a_{NTBI} + n_{spk,57} \cdot {}^{57}a_{spk,57} + n_{spk,58} \cdot {}^{57}a_{spk,58} \tag{S-3}$$

$$n(^{58}Fe) = n_{NTBI} \cdot {}^{58}a_{NTBI} + n_{spk,57} \cdot {}^{58}a_{spk,57} + n_{spk,58} \cdot {}^{58}a_{spk,58} \tag{S-4}$$

The isotope ratio ${}^{57/56}R = \frac{n(^{57}Fe)}{n(^{56}Fe)}$ in the isotope diluted sample can be expressed using Eqns. (S-2) and (S-3),

$${}^{57/56}R = \frac{n_{NTBI} \cdot {}^{57}a_{NTBI} + n_{spk,57} \cdot {}^{57}a_{spk,57} + n_{spk,58} \cdot {}^{57}a_{spk,58}}{n_{NTBI} \cdot {}^{56}a_{NTBI} + n_{spk,57} \cdot {}^{56}a_{spk,57} + n_{spk,58} \cdot {}^{56}a_{spk,58}} \tag{S-5}$$

Likewise, Eqns. (S-2) and (S-4) can be used to express the isotope ratio ${}^{58/56}R = \frac{n(^{58}Fe)}{n(^{56}Fe)}$ in the sample,

$${}^{58/56}R = \frac{n_{NTBI} \cdot {}^{58}a_{NTBI} + n_{spk,57} \cdot {}^{58}a_{spk,57} + n_{spk,58} \cdot {}^{58}a_{spk,58}}{n_{NTBI} \cdot {}^{56}a_{NTBI} + n_{spk,57} \cdot {}^{56}a_{spk,57} + n_{spk,58} \cdot {}^{56}a_{spk,58}} \tag{S-6}$$

Eqns. (S-5) and (S-6), respectively, can be transformed to yield $n_{spk,58}$.

$$n_{spk,58} = n_{NTBI} \cdot \frac{{}^{57}a_{NTBI} - {}^{56}a_{NTBI} \cdot {}^{57/56}R}{{}^{56}a_{spk,58} \cdot {}^{57/56}R - {}^{57}a_{spk,58}} + n_{spk,57} \cdot \frac{{}^{57}a_{spk,57} - {}^{56}a_{spk,57} \cdot {}^{57/56}R}{{}^{56}a_{spk,58} \cdot {}^{57/56}R - {}^{57}a_{spk,58}} \tag{S-7}$$

$$n_{spk,58} = n_{NTBI} \cdot \frac{{}^{58}a_{NTBI} - {}^{56}a_{NTBI} \cdot {}^{58/56}R}{{}^{56}a_{spk,58} \cdot {}^{58/56}R - {}^{58}a_{spk,58}} + n_{spk,57} \cdot \frac{{}^{58}a_{spk,57} - {}^{56}a_{spk,57} \cdot {}^{58/56}R}{{}^{56}a_{spk,58} \cdot {}^{58/56}R - {}^{58}a_{spk,58}}$$

(S-8)

By equating Eqn. (S-7) and (S-8), the molar ratio of $n_{spk,57}$ to n_{NTBI} can be obtained,

$$\frac{n_{spk,57}}{n_{NTBI}} = \frac{\alpha - \beta}{\gamma - \delta} \qquad \text{(S-9)}$$

and the amount ratio of $n_{spk,58}$ to n_{nat} can also be calculated:

$$\frac{n_{spk,58}}{n_{NTBI}} = \frac{\alpha\gamma - \beta\delta}{\gamma - \delta} \qquad \text{(S-10)}$$

Variables α, β, γ, δ in Eqns. (S-9) and (S-10) are defined by the following equations:

$$\alpha = \frac{{}^{58}a_{NTBI} - {}^{56}a_{NTBI} \cdot {}^{58/56}R}{{}^{56}a_{spk,58} \cdot {}^{58/56}R - {}^{58}a_{spk,58}} \qquad \text{(S-11)}$$

$$\beta = \frac{{}^{57}a_{NTBI} - {}^{56}a_{NTBI} \cdot {}^{57/56}R}{{}^{56}a_{spk,58} \cdot {}^{57/56}R - {}^{57}a_{spk,58}} \qquad \text{(S-12)}$$

$$\gamma = \frac{{}^{57}a_{spk,57} - {}^{56}a_{spk,57} \cdot {}^{57/56}R}{{}^{56}a_{spk,58} \cdot {}^{57/56}R - {}^{57}a_{spk,58}} \qquad \text{(S-13)}$$

$$\delta = \frac{{}^{58}a_{spk,57} - {}^{56}a_{spk,57} \cdot {}^{58/56}R}{{}^{56}a_{spk,58} \cdot {}^{58/56}R - {}^{58}a_{spk,58}} \qquad \text{(S-14)}$$

Rearrangement of Eqn. (S-10) yields

$$n_{NTBI} = n_{spk,58} \cdot \frac{\gamma - \delta}{\alpha\gamma - \beta\delta} \qquad \text{(S-15)}$$

In Eqn. (S-15), all the isotopic abundances and isotope ratios can be measured by thermal ionization mass spectrometry (TIMS). With the amount of ^{58}Fe spike added into the filtrate being known, the amount of NTBI in the filtrate can be calculated.

1.2 Derivation of equations used for calculation of $NTBI_{exch}\%$

With $n(NTA \cdot {}^{57}Fe_{spk})$ as the amount of ^{57}Fe spike added into the serum in moles to mimic NTBI and $n(NTA \cdot Fe)$ as the amount of natural iron added in excess into the serum to saturate vacant binding sites of transferrin, the amount of NTBI that has undergone species conversion with transferrin $n(NTBI_{exch})$ equals the amount of transferrin bound ^{57}Fe spike $n(Tf \cdot {}^{57}Fe_{spk})$,

$$n(NTBI_{exch}) = n(Tf \cdot {}^{57}Fe_{spk}) \tag{S-16}$$

Because of species conversion, the amount of exchanged transferrin bound iron $n(Tf \cdot Fe_{exch})$ is also equal to the amount of transferrin bound ^{57}Fe spike,

$$n(Tf \cdot Fe_{exch}) = n(Tf \cdot {}^{57}Fe_{spk}) \tag{S-17}$$

After species conversion, the amount of ^{57}Fe spike in the filtrate $n({}^{57}Fe_{spk})$ is equal to the amount of ^{57}Fe spike added into the serum minus the amount of ^{57}Fe spike exchanged,

$$n({}^{57}Fe_{spk}) = n(NTA \cdot {}^{57}Fe_{spk}) - n(NTBI_{exch}) \tag{S-18}$$

and the amount of natural iron in the filtrate $n(Fe_{nat})$ is equal to the amount of natural iron added into the serum plus the amount of exchanged transferrin bound iron:

$$n(Fe_{nat}) = n(NTA \cdot Fe) + n(Tf \cdot Fe_{exch}) \tag{S-19}$$

The amount ratio R of ^{57}Fe spike to natural iron in the filtrate

$$R = \frac{n({}^{57}Fe_{spk})}{n(Fe_{nat})} \tag{S-20}$$

can then be calculated from measured iron isotope ratios of the filtrate following isotope dilution mass spectrometry (IDMS) principles.

Substitution of Eqn. (S-18) and Eqn. (S-19) into Eqn. (S-20) yields

$$R = \frac{n(NTA \cdot {}^{57}Fe_{spk}) - n(NTBI_{exch})}{n(NTA \cdot Fe) + n(Tf \cdot Fe_{exch})} \tag{S-21}$$

and substitution of Eqn. (S-16) and Eqn. (S-17) into Eqn. (S-21) yields

$$R = \frac{n(NTA \cdot {}^{57}Fe_{spk}) - n(NTBI_{exch})}{n(NTA \cdot Fe) + n(NTBI_{exch}))} \tag{S-22}$$

Which is rearranged to yield

$$n(NTBI_{exch}) = \frac{n(NTA \cdot {}^{57}Fe_{spk}) - R \cdot n(NTA \cdot Fe)}{R + 1} \tag{S-23}$$

The percentage of NTBI that has undergone species conversion with transferrin $NTBI_{exch}\%$ is expressed relative to the amount of ^{57}Fe spike added into the serum as $NTA \cdot {}^{57}Fe_{spk}$ to mimic NTBI,

$$NTBI_{exch}\% = \frac{n(NTBI_{exch})}{n(NTA \cdot {}^{57}Fe_{spk})} \tag{S-24}$$

Substitution of Eqn. (S-23) into Eqn. (S-24) yields finally:

$$NTBI_{exch}\% = \frac{n(NTA \cdot {}^{57}Fe_{spk}) - R \cdot n(NTA \cdot Fe)}{n(NTA \cdot {}^{57}Fe_{spk}) \cdot (R+1)} \tag{S-25}$$

In Eqn. (S-25), the isotope ratio R can be measured by TIMS. With $n(NTA \cdot {}^{57}Fe_{spk})$ and $n(NTA \cdot Fe)$ being known, the percentage of NTBI that has undergone species conversion can be calculated.

1.3 Inclusion and exclusion criteria of participants

Inclusion criteria:

- Apparently healthy male adults (21 – 55 years old)
- No indications of iron overload or iron deficiency
- Haemoglobin concentration: 12.9 – 17.0 g/dL
- Serum ferritin concentration: 20 – 300 µg/L
- Transferrin saturation (TSAT): 20 – 50%
- C-reactive protein (CRP): < 5 mg/L

Exclusion criteria:

- Blood donation or significant blood loss within the past 4 months
- Regular intake of iron and other nutrient supplements within the past 4 months
- Regular intake of medication
- Acute or recent inflammatory symptoms
- Chronic gastrointestinal disorders or known chronic or metabolic disease
- Anaemia and abnormal iron status parameters indicative of iron overload or iron deficiency

References

T. Walczyk, L. Davidsson, N. Zavaleta and R. F. Hurrell, Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal, *Fresenius J. Anal. Chem.*, 1997, **359**, 445-449.