Web Appendix - I

Details of the calculations used to arrive at physiological iron requirement is as follows –

Basal Iron Loss

The weight standardized basal iron loss reported in adult men from Seattle, Venezuela and South African Indians was used [1], since data on basal iron loss were not available for children and adolescents. The mean basal loss of iron reported was 14 µg/kg body weight/d with a standard deviation (SD) of 4.1 µg/kg body weight/d. To calculate the SD of body iron loss, the SD of the basal iron loss (per kg) was combined with the SD of reported body weight (kg) of children and adolescents, as the variance of the product of two independent random variables.

$$\sigma_{xy}^2 = \sigma_x^2\sigma_y^2 + \sigma_x^2\mu_y^2 + \sigma_y^2\mu_x^2 \qquad (1)$$

where $\sigma_x, \sigma_y, \mu_x$ and μ_y are SD and mean respectively, of *x* (basal loss) and *y* (body weight).

Increment in Blood Volume and Hemoglobin Mass

To estimate the increment of blood volume per day, sex- and age-specific blood volume data were extracted for children aged up to 17y from earlier studies [2,3], and regressed on age, ignoring sex for children <10y, since sexual dimorphism before adolescence is likely to be low. For adolescents, sex-specific regression coefficients were estimated and applied in subsequent equations. The estimated regression coefficient (β_{bv})was further adjusted by the ratio of the mean (50th percentile) of the standard weight for age [4] with the mean weight reported in earlier studies [2, 3] at that age, by the following equation to obtain the annual increase in blood volume for age:

$$\Delta \mathrm{bv}_t = \mathrm{R}_t\beta_{bv};\ R_t = \frac{[\mathrm{weight}_{standard}]_t}{[\mathrm{weight}_{Morse,Russell}]_t} \qquad (2)$$

where $\Delta \mathrm{bv}_t$ was the weight adjusted annual increase in blood volume; β_{bv} was the annual increment of blood volume estimated from references 2 and 3; the subscript *t* refers to age; $\mathrm{weight}_{standard}$ represents standard weight; the WHO age and sex specific standard was used for children and adolescents 1-17y of age; $\mathrm{weight}_{Morse,Russell}$ represents the average weight for age obtained by combining data from references 2 and 3.

The variation of Δbv_t was derived by combining the variation of the weight for age and β_{bv} using Equation 1 above. For adolescents, sex-specific regression coefficients (β_{bv}) were estimated and applied in subsequent equations. The specific average requirement of iron due to the increase in blood volume (Iron_{bv}) or increment in Hb mass for any given age was derived by following equation

$$\mathrm{Iron}_{bv} = \Delta bv_t \times [\text{Hb concentration}] \times [\text{Iron content in Hb}] \quad (3)$$

In this equation, the Hb concentration was taken as 110, 115 and 120 g/L for <5, 5-11 and >11 ages respectively [5] and the iron content of Hb was assumed to be 3.39 mg/g [6]. The SD of the Iron_{bv} was estimated from the SD of Δbv_t from equation (3).

Non-storage Tissue Iron

The non-storage tissue iron was calculated as the product of weight gain/d and iron deposited within that weight gain. The mean weight gain/d and its variance were estimated from annual body weights and converting the yearly change of weight (mean and variance) to a daily change. For 1-9y children, the non-storage tissue iron was calculated as the product of the total body iron content (38 mg/kg) in children aged 1-9y [7] and the proportion of this total body iron content which is non-storage content, assumed to be 14% [8]. From 10y onward, since muscle development is significant, the muscle content of iron was taken to represent non-storage tissue iron deposition (as myoglobin). This has been found to be 26 mg/kg and independent of the dietary iron intake [9]. Assuming that 40% of the increment in body weight is due to skeletal muscle growth [10], a value of 10 mg/kg body weight increase was assumed for non-storage tissue iron deposition in adolescents.

Iron Required for Storage

The mean daily requirement for storage iron for children and adolescents was calculated based on the assumption that 12% of the increment in tissue iron deposition and Hb mass enters into body storage [6], which is a reasonable assumption. Alternatively, the storage iron could also be directly calculated as proportion of the total body iron (14%) at each age [11]. In a sensitivity analysis, using these assumptions resulted in a minimal difference in the final physiological requirement, and therefore, the former method, that was adopted by the Institute of Medicine (IOM) [6], was used in the present analysis.

References

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