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Development and validation of a dietary iron score for screening populations at risk for inadequate iron intake

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ABSTRACT

Introduction: Inadequate iron intake is a determinant of iron deficiency. A simplified tool for dietary assessment is needed. This study aimed to develop a dietary iron scoring system (DISS) and to determine the validity of the dietary iron score (DIS) for screening populations at risk for inadequate iron intake. **Methods:** A three-step process was undertaken to develop the DISS, namely (1) iron score (IS) for each food was constructed based on its iron content per 100 g, adjusted for heme content equivalence; (2) the predicted modifying effect (PME) was formulated based on either enhancing or inhibiting effects of dietary constituents; (3) the DIS of a meal was obtained by multiplying the total IS and the PME of that meal. The validity of the DIS for screening populations at risk for inadequate iron intake was determined against absorbable iron calculated by the Hallberg & Hulthen algorithm. A probability of adequacy of absorbable iron intake of 0.75 was used as a cutoff in defining the population at risk. **Results:** There was a significant correlation between the absorbable iron and DIS (r=0.34, p<0.001). Using the Receiver Operating Characteristic (ROC) curve , three cutoffs of DIS, namely 5, 6 and 7, had comparable results. However, sensitivity (82.9%) and specificity (50.0%) was the best for DIS cutoff of 7. **Conclusion:** The proposed DISS is potentially a field-friendly tool for screening populations at risk for inadequate iron intake. Further verifications are needed, using more complete dietary data.

Keywords: Iron, dietary iron score, screening tool

INTRODUCTION

Iron deficiency has been recognised as one of the most significant public health problems in the world. The World Health Organization (WHO) estimated that 42%, 49% and 50% of children under 5 years, non-pregnant and pregnant women, respectively were anaemic (WHO, 2015). About half of the anaemic cases in developing countries were associated with iron deficiency (Erick *et al.*, 2009). Poor iron status is associated with reduced work capacity, lowered immunity, and reduced cognition (WHO, 2001). Much effort has been made to prevent and control this nutritional problem throughout the world.

A key element for programmes to alleviate nutrient deficiency is to have an appropriate assessment tool, which is simple, practical and low cost. Determination of iron intake is useful

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for assessing the adequacy of dietary iron to meet iron requirements. The critical issue concerning diet and iron status is not the total amount of iron ingested but rather, the amount of iron available for absorption. Importantly, absorption of iron is highly variable, depending not only on the iron status of the individual, but also on other factors present in the diet that enhance or inhibit its absorption. Iron absorption can vary more than ten-folds at fixed iron content (Hallberg & Hulthen, 2000). Thus, the amount of bioavailable iron is most relevant for determining whether or not iron requirements are met in a population.

Twenty-four hours recall or record of food intake for one or more days, or a food frequency questionnaire is commonly used in investigating the risk or etiology of micronutrient deficiencies. Even though these methods provide detailed data, they are time consuming and require skilled workers. In addition, data processing to estimate the amounts of nutrient intake is a complicated and tedious procedure. Consequently, the dietary diversity score (DDS) was introduced as a simplified dietary assessment tool for non-nutritionists and other lay users. It involves a summation of the number of food items or food groups consumed over a specified period of time (1-7 days or up to 15 days) (Ruel, 2003). It was found that it was not complicated to train field staffs to obtain information on dietary diversity; it was not invasive and burdensome for the respondents, and not time consuming (FANTA, 2002). Several studies consistently showed positive correlations between DDS and micronutrient adequacy (Foote *et al.*, 2004; Kennedy *et al.*, 2007; Moursi *et al.*, 2008; Savy *et al.*, 2008; Steyn *et al.*, 2006). Therefore, the DDS concept is an appealing approach for assessing micronutrient adequacy. However, the correlation coefficient between DDS and micronutrient adequacy has been found quite low (r=0.30-0.40), and presently there is no consensus on the measurement components of DDS, such as classification of food groups, scoring system, minimum portion size of specific foods for inclusion, and DDS cutoffs (Ruel, 2003). Moreover, DDS does not take into account the bioavailability of micronutrients in aggregating food groups and performing the scoring system. This may lead to uncertainty in assessing adequacy of micronutrients, especially iron, zinc and vitamin A. Therefore, development of a dietary iron scoring system which considers iron bioavailability would provide a more reliable dietary assessment tool to identify populations at risk for iron deficiency.

This study was designed to develop and validate a dietary iron scoring system, which is an intentionally simplified dietary assessment method for screening adequacy of iron intake. The proposed tool also takes into account enhancers and inhibitors of iron absorption.

MATERIALS AND METHODS

Development of dietary iron scoring system

The development of a dietary score for iron was based on the principle that bioavailability of iron from foods depends on the form of iron (heme or non-heme) and other constituents present in foods. While heme iron is readily absorbable, non-heme iron absorption is determined by the presence of other food constituents. Dietary constituents which modify the absorption of iron fall into two main groups – iron absorption enhancers and inhibitors. The development of the proposed dietary iron scoring system (DISS) consisted of three main parts, namely (1) adjusting the scores for iron contents for heme/

non-heme iron contents (iron score; IS), (2) deriving scores for iron absorption modifying effects (modifying score; MS) and (3) calculating scores for availability of dietary iron in a meal (dietary iron score; DIS).

Iron score (IS)
Heme iron is present in animal food sources, including red meat, organ meat, poultry and fish in varying proportions. The higher the heme iron content, the higher the iron bioavailability. The rest of the iron in these foods is in the non-heme form. Although classified as animal food sources, iron in eggs and milk are non-heme iron and less bioavailable than the iron in meat sources (Callender, Marney & Warner, 1970). In plant sources, iron is in various chemical forms and collectively referred to as non-heme iron. Therefore, iron score in this study is based on the total iron content adjusted for its availability based on the proportion of heme in the food.

Scores for iron content
Eight hundred and nine food items listed in INMUCAL-Nutrient 4.1 database (Institute of Nutrition, 2007) were classified into eight groups, namely meat, milk, egg, cereals, tubers, legumes & nuts, vegetables and fruits. Foods having similar iron contents were then aggregated into 56 subgroups. The median value of iron content of each group/subgroup was computed. The minimum score of 1 was set for iron content below the tenth percentile (corresponds to an iron content of 0.44 mg/100 g, rounding to 0.5 mg). Since the average Thai recommended dietary intake (MOPH Thailand, 2003) of iron from the age of 6 years upwards is on average of 10 mg, and the bioavailability of habitual Thai diets is 10% of total iron (Hallberg *et al.*, 1974), thus, a score of 1 was given for each increment of 1 mg iron (Table 1).

Table 1. Iron scores (IS) according to amount of iron in foods

Iron score (IS)	*Iron content in foods, mg/ 100 g*
1	< 0.50
2	0.51-1.50
3	1.51-2.50
4	2.51-3.50
5	3.51-4.50
6	4.51-5.50
7	5.51-6.50
8	6.51-7.50
9	7.51-8.50
10	8.51-9.50
11	9.51-10.50
12	10.51-11.50
13	11.51-12.50
14	12.51-13.50
15	13.51-14.50
16	14.51-15.50
17	15.51-16.50
18	16.51-17.50
19	17.51-18.50
20	18.51-19.50
21	19.51-20.50

Adjusting for heme content in foods by weighting scores
Absorption of both heme and non-heme iron depends on the iron status of individuals. Based on the regression equations for heme and non-heme iron absorption related to iron status (Hallberg, Hulthen & Gramatkovski, 1997), the ratio of iron absorption of heme iron to non-heme iron at an average iron store of 500 mg (the average of estimated iron store based on serum ferritin level in Thai school children) was set at 3.5:1. This ratio was used as basis for deriving a weighting score for conversion of heme iron to non-heme iron. Then, the weighting score for adjusted heme content in foods was calculated by the derived equation of $[(2.5 \times \%\ \text{heme}) + 100]/100$. Due to its wide content range, heme iron contents in animal food sources were sub-grouped according to the percentage of heme iron based on literature values (Napatthalung, 2000). In this way, a calculated weighting was determined for each subgroup. Table 2 presents the calculated weightings and accordingly, the assigned weighting

score after adjusting for heme and non-heme iron contents. These weighting scores were used to multiply the iron contents in foods to derive the final iron score for various foods.

Modifying score (MS) and predicted modifying effect (PME)

The total absorbable iron in a meal is the net result of interaction between the form of iron and iron absorption modifiers in a meal. It is not known whether these effects are additive or multiplicative. Therefore, the following steps were taken to obtain the MS for each food item. The net modifying effects of a meal were derived based on a linear regression of MS on calculated modifying effects of the meal, that were derived by using the Hallberg & Hulthen algorithm of all possible combinations of foods (Hallberg & Hulthen, 2000). This prediction equation, called predicted modifying effect (PME) was then used to derive the dietary iron scores of meals. Details of these steps are as follow:

I. Compilation of contents of vitamin C, phytate, calcium, and tannin

Since the extent of modifying effect of inhibitors/enhancers is associated with the amount ingested, and as consumption size varies according to age, it is more accurate to derive the MS according to the target age group. In this study, school age children were chosen. Lists of foods commonly consumed by children aged 6-12 years were identified from the Thai Food Consumption Survey 2003-2004 (National Bureau of Agricultural Commodity and Food Standards, 2007), followed by reduction to only food items for which the percentage of consumption was more than 50.0%. Contents of vitamin C, phytate, calcium and tannin were obtained from INMUCAL-Nutrient 4.1 and other sources (Chansuwan, 2005; Charoensiri & Kongkachuichai, 2008; Harland & Harland, 1980; Ma *et al.*, 2005; Ravindran, Ravindran & Sivalogan, 1994; Reddy, 2002; Somsub *et al.*, 2008; Suttikomin, 2002). When data on these contents were not available, the values were estimated from other foods that have similar characteristics.

II. Calculation of the inhibiting or enhancing effect of dietary modifiers by food item and portion size

Since the absorption of non-heme iron depends on the co-presence of factors that enhance or inhibit iron absorption, the score for the net modifying effect associated with each food item was required. Hallberg and Hulthen (2000) provided algorithms for calculating absorption ratio (AR) for various food constituents (factors). The AR value was derived directly from the measured absorption value when the factor is present, otherwise, the absorption was estimated as follows:

Table 2. Weighting scores for adjusting heme iron content in foods to non-heme iron

Foods by percentage of heme iron	*Calculated weighting*	*Weighting score*
Plant, egg and milk	0.00	1.0
Animal sources:		
<10% heme	< 1.25	1.0
10-30%	1.25-1.75	1.5
31-50%	1.75-2.25	2.0
51-70%	2.25-2.75	2.5
71-90%	2.75-3.25	3.0

Note: Calculated weighting was derived by using the formula; [(2.5 × % heme) + 100]/100

Median weighting score is derived from calculating the weighting score for each range of heme percentage

Vitamin C-factor
$= 1 + (0.01 \times \text{Vit C}) + \log(\text{phytate-P} + 1) \times 0.01 \times 10^{0.8875\times \log(\text{Vit C}+1)}$

Meat, fish poultry (MFP)-factor
$= (1 + 0.01\text{MFP}) \times 10^{0.4515-[0.715-0.1825\times\log(1+\text{Vit C})]\times \log(1+\text{Tannin})}$

Phytate-factor
$= 10^{(-0.30 \times \log(1+\text{mg phytate-P}))}$

Calcium-factor
$= 0.4081 + (0.5919/1 + 10^{-[2.022-\log(\text{Calcium}+1)]\times 2.919})$; where calcium is ≤ 50 mg, calcium-factor is assumed to be 1.

Tannin-factor
$= (1 + 0.01\text{MFP}) \times 10^{0.4515-[0.715-0.1825\times\log(1+\text{Vit C})]\times \log(1+\text{Tannin})}$; the factor should be ≤ 1, corrected to 1 if it is not.

Since the inhibiting or enhancing effect is dependent on the amount of the specific food eaten, the portion size of each food item consumed was required. The median values for portion size were obtained from the Thai Food Consumption Survey 2003-2004 (National Bureau of Agricultural Commodity and Food Standards, 2007), based on dietary intake data of children aged 6-12 years. The portion size used for estimating the modifying effect of dietary factors were: small = median/2; medium size = median; and large size = median × 2.

When more than one enhancing or inhibiting factor is present in a food item, the AR for that food is the product of AR for each of the factors estimated separately for enhancing and inhibiting factors. AR for enhancers (AR-Enhancer) included vitamin C-factor and MFP-factor, while AR for inhibitors (AR-Inhibitor) included phytate-factor, calcium-factor and tannin-factor. Finally, the MS for each food item by portion size was calculated as follows:

$$MS = [(\text{AR-Enhancer} - 1) + (\text{AR-Inhibitor} - 1)] \times 10^*$$

*This is an arbitary number to enable easy counting of the score

III. Determination of the PME of dietary factors in a meal pattern

Although it is known that when dietary factors in individual foods are combined in a meal leading to changes in iron bioavailability, there is no generally accepted model for estimating the results from such interactions. Therefore, summing up the modifying scores of enhancers and inhibitors is practical, but will not be meaningful. This study used a regression approach to obtain the predicted modifying effects of iron modifiers in a meal by adding the sum modifying scores of a meal.

In order to determine the PME, hypothetical meals based on all known dietary patterns were formulated. These meals represent various combinations of the quantity and extent of modifying effects of food items present in a meal. The foods selected in designing the hypothetical meals were grouped according to habitual dietary patterns of Thai diets, as follows:

1) Rice: Rice represents the staple food of the dietary pattern in Thailand.
2) Iron source foods: Six food characteristics representing levels of iron content, percent heme and concurrent modifiers were chosen (e.g. extremely high iron & high heme content, high iron & moderate heme content, moderate iron & high heme content, low iron & moderate heme content, non-heme iron with inhibitor, non-heme iron without inhibitor).
3) Vegetables: There are three types of vegetables defined according to the AR-Enhancer and AR-Inhibitor estimations covering the range of vegetables commonly consumed in Thai diets (e.g. low/no enhancer & high inhibitor, low/no enhancer & moderate inhibitor, moderate enhancer & low inhibitor).
4) Fruits: Similarly, four characteristics of fruits were chosen according to the AR-Enhancer and AR-Inhibitor values in fruits commonly consumed

Table 3. Comparison of correlation coefficients for linear relationships derived from four meal patterns

Meal pattern	*n*	*Constant*	*Slope*	R^2
Rice-based meal with iron source	204	0.703	0.035	0.71
Meals without rice	198	0.831	0.057	0.75
Rice-based meal without iron source	204	0.611	0.030	0.79
All meals pooled	600	0.764	0.042	0.72

(e.g. high enhancer & moderate inhibitor, moderate/low inhibitor & moderate inhibitor, moderate enhancer & less/no inhibitor, low enhancer & low inhibitor).

5) Milk: Due to the calcium content in milk and the fact that milk is now commonly consumed, especially among school-aged children, hypothetical meals were designed to include those that are taken with or without milk.

Meal combinations were then formulated to represent all known combinations of the food groups mentioned above. Nine groups of combinations were designed as following:

a. Rice + one iron source + one vegetable + one fruit (72 meal combinations)
b. Rice + one iron source + one vegetable + one fruit + milk (72 meal combinations)
c. Rice + one iron source (6 meal combinations)
d. Rice + one iron source + one vegetable (24 meal combinations)
e. Rice + one iron source + one fruit (18 meal combinations)
f. Rice + one iron source + milk (6 meal combinations)
g. One iron source + milk (6 meal combinations)
h. All meal in group a-f excluded rice (198 meal combinations)
i. All meal in group a-g excluded iron source (204 meal combinations)

Finally, 600 hypothetical meals were designed to derive the prediction equation for iron modifying effects in meals.

IV. Deriving the prediction equations of modifying effects for different types of meals

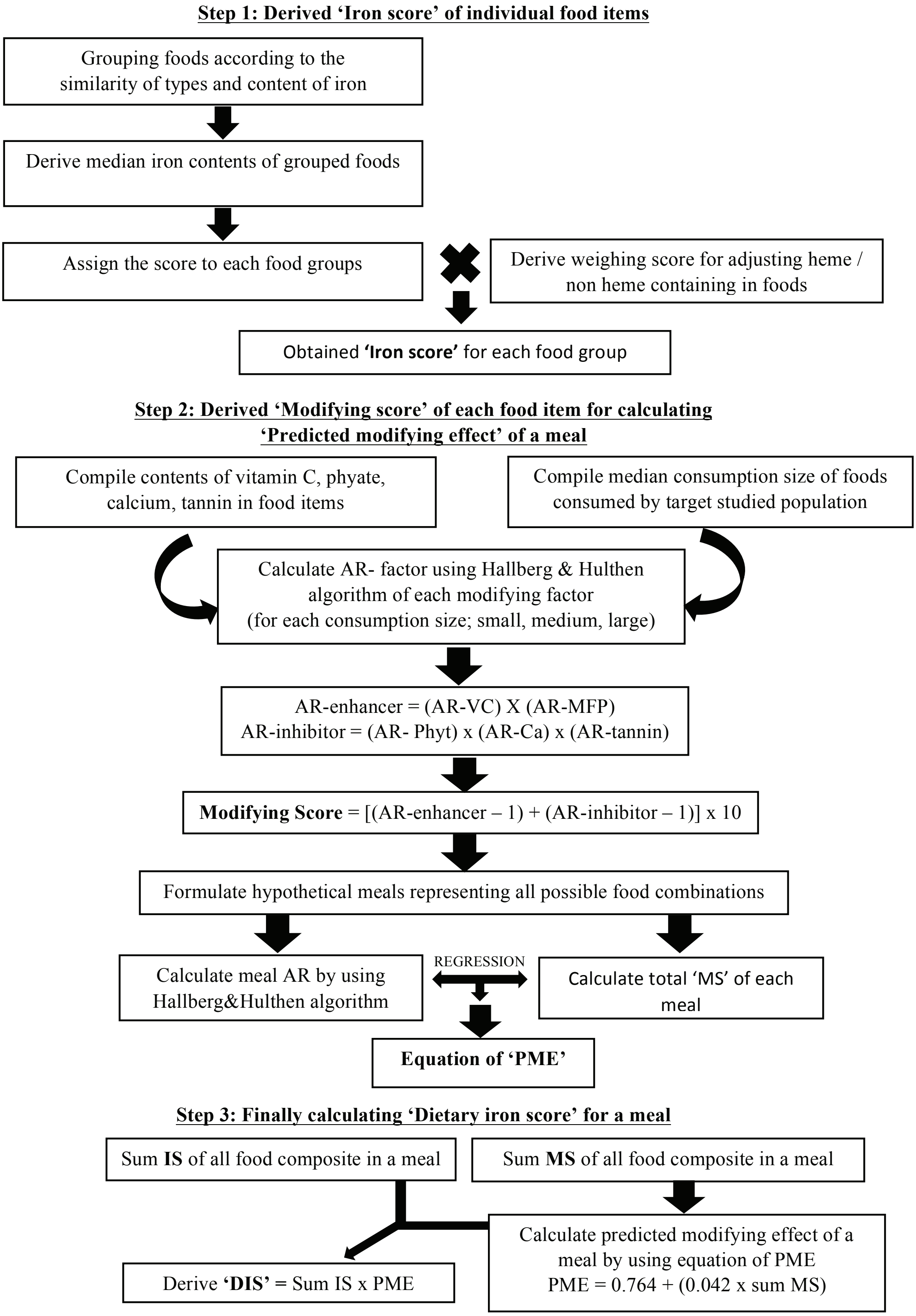
According the 600 hypothetical meals, four different sets of meal patterns were examined, namely, (1) rice-based meals with iron source, (2) meals without rice, (3) rice-based meal without iron source, and (4) all meals. The AR of each meal was calculated by using the Hallberg & Hulthen algorithm (Hallberg & Hulthen, 2000) and the modifying score of each food item in a meal as given in step II above. The comparison of the various regression parameters using different meal compositions as described above are shown in Table 3. Since the slope and correlation coefficient of the four regression lines were similar, it was decided that the equation for the pooling of all meals could be used for any meal patterns. Thus, the equation for the PME is:

$$PME = 0.764 + (0.042 \times \text{sum MS})$$

Deriving total DIS for a meal and per day

The final DIS for each meal was derived by multiplying the IS and PME. For comparison of DIS of individuals, DIS of all meals consumed in a day was summed to obtain the dietary score per day.

A summary of the steps involved in developing the DISS is shown in the schematic below.


Step 1: Derived 'Iron score' of individual food items
Grouping foods according to the similarity of types and content of iron
Derive median iron contents of grouped foods
Assign the score to each food groups
Derive weighing score for adjusting heme / non heme containing in foods
Obtained 'Iron score' for each food group
Step 2: Derived 'Modifying score' of each food item for calculating 'Predicted modifying effect' of a meal
Compile contents of vitamin C, phyate, calcium, tannin in food items
Compile median consumption size of foods consumed by target studied population
Calculate AR- factor using Hallberg & Hulthen algorithm of each modifying factor (for each consumption size; small, medium, large)
AR-enhancer = (AR-VC) X (AR-MFP)
AR-inhibitor = (AR- Phyt) x (AR-Ca) x (AR-tannin)
Modifying Score = [(AR-enhancer – 1) + (AR-inhibitor – 1)] x 10
Formulate hypothetical meals representing all possible food combinations
Calculate meal AR by using Hallberg&Hulthen algorithm
REGRESSION
Calculate total 'MS' of each meal
Equation of 'PME'
Step 3: Finally calculating 'Dietary iron score' for a meal
Sum IS of all food composite in a meal
Sum MS of all food composite in a meal
Calculate predicted modifying effect of a meal by using equation of PME
PME = 0.764 + (0.042 x sum MS)
Derive 'DIS' = Sum IS x PME

Validation of the developed DIS with Hallberg & Hulthen algorithm and its use in assessing the risk of inadequate iron intake

Dietary data used in the present study were from the Project entitled 'Efficacy of multiple micronutrients fortified soup-based instant noodles in school children, Northeast Thailand', conducted between 2003-2004 (Winichagoon *et al.*, 2006). Briefly, this is a randomised placebo-controlled trial comparing biochemical parameters, growth, morbidity and cognition of children receiving micronutrient-fortified (vitamin A, iron, iodine and zinc) vs non-fortified lunch in school for 32 weeks. Dietary intakes of stunted and non-stunted (ratio 1:4) children aged 6-12 years (n=230) were assessed using one 24-hour recall by trained interviewers. Attempt was made to include both week-day and week-ends in the dietary assessment.

Determination of probability of adequacy of iron intake

Probability of adequacy (PA) is the probability that an individual's usual intake is greater than the nutrient requirement. This is determined by dividing the difference between the estimated nutrient intake and estimated average requirement (EAR) with the standard deviation of the reference requirements. Since the frequency distribution of iron requirement is not a normal pattern, the concept of PA could not be applied directly (Institute of Medicine, 2000). The Institute of Medicine (IOM) suggested using the table of probability of inadequacy (PI). A matrix for probability of inadequate (PI) iron intake for children aged 4-8 years and 9-13 years was then constructed. The recommended iron intakes in these tables were based on iron absorption of 18.0%. Therefore, the intake values were converted to absorbed iron by multiplying the recommended intakes by 0.18. Finally, the PI was transformed to PA by subtracting the values from one.

Absorbable iron from the dietary intake data of school children was calculated using the Hallberg & Hulthen algorithm (2000) and transformed to PA according to this guideline. For validation of the DIS, this PA was used for comparison of its performance in identifying populations at risk for iron inadequacy.

Validation of the 'DIS' against the 'absorbable iron' algorithm (Hallberg & Hulthen, 2000) as a reference method

The agreement of the two methods in classifying a population by the adequacy of iron intake was performed using Kappa statistics. The Receiver Operating Curve (ROC) was used to derive the cutoff for the DIS for defining risk of inadequacy of iron intake. Sensitivity and specificity were calculated for different cutoffs of dietary iron score to identify populations at risk for iron inadequacy using PA cutoffs at 0.75.

RESULTS

Assessing risk of inadequacy of iron intake using DIS versus absorbable iron derived by the Hallberg & Hulthen algorithm

Absorbable iron derived using the Hallberg & Hulthen algorithm was used as a reference method for validation of the DIS. First, the risk of inadequacy of iron intake was determined by the PA approach. A PA of '0' indicates 100% risk of inadequacy of iron intake, while PA of '1' indicates zero risk of inadequate iron intake. In other words, the higher the PA, the lower is the risk of inadequacy. Applying this approach using the data of Winichagoon *et al.* (2006), it was found that about 63.0% of the school children were likely to have inadequate iron intake, i.e., PA = 0 (expressed as absorbable iron). Cumulatively, only about eight percent of them had PA of iron intake above 0.55.

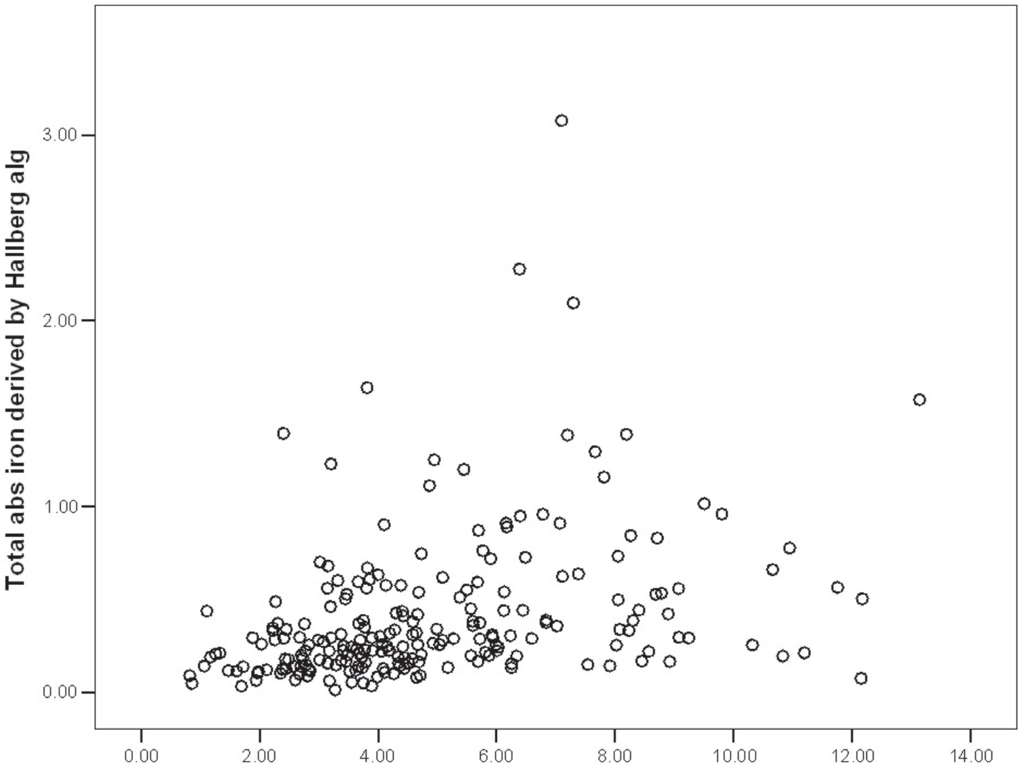


Figure 1. Scatter plot of absorbable iron calculated by the Hallberg & Hulthen algorithm and dietary iron score (*r*=0.34, *p*<0.001)

Correlation between dietary iron score and absorbable iron

Dietary iron score and absorbable iron estimated by the Hallberg & Hulthen algorithm were determined. Figure 1 shows the scatter plot between DIS and absorbable iron calculated from the Hallberg & Hulthen algorithm. The correlation between absorbable iron and DIS was found to be significant (*r*= 0.34, *p*<0.001).

Determination of the cutoffs for DIS to identify populations at risk of inadequate iron intakes

The cutoff of PA of 0.50, was recommended for use to define individuals at risk of nutrient inadequacy (Institute of Medicine, 2000) while PA 0.75 had been used as an alternative (Kennedy *et al.*, 2007). Theoretically, a risk population identified by using PA cutoff of 0.50 is more likely to have a higher risk of iron deficiency than those identified by using PA cutoff of 0.75. Different cutoffs of dietary iron score ranging from 1-12 were applied and compared to the PA derived from absorbable iron intake to identify populations at risk of iron inadequacy (PA<0.75). The ROCs of 12 DIS cutoffs, including ranges of values (1-12), were examined. The three DIS cutoffs of 5, 6, and 7 were considerably higher than the rest of the 12 cutoffs (Figure 2). Based on the area under the curve (AUC), this result indicates that the cutoff of 5 was good while the other two, namely 6 and 7 were fair (Tape, 2010). While these three cutoffs were considered reasonable cutoffs, DIS cutoff at 7 provides the optimal performance based on sensitivity 82.9% and specificity 50.0% (Table 4).

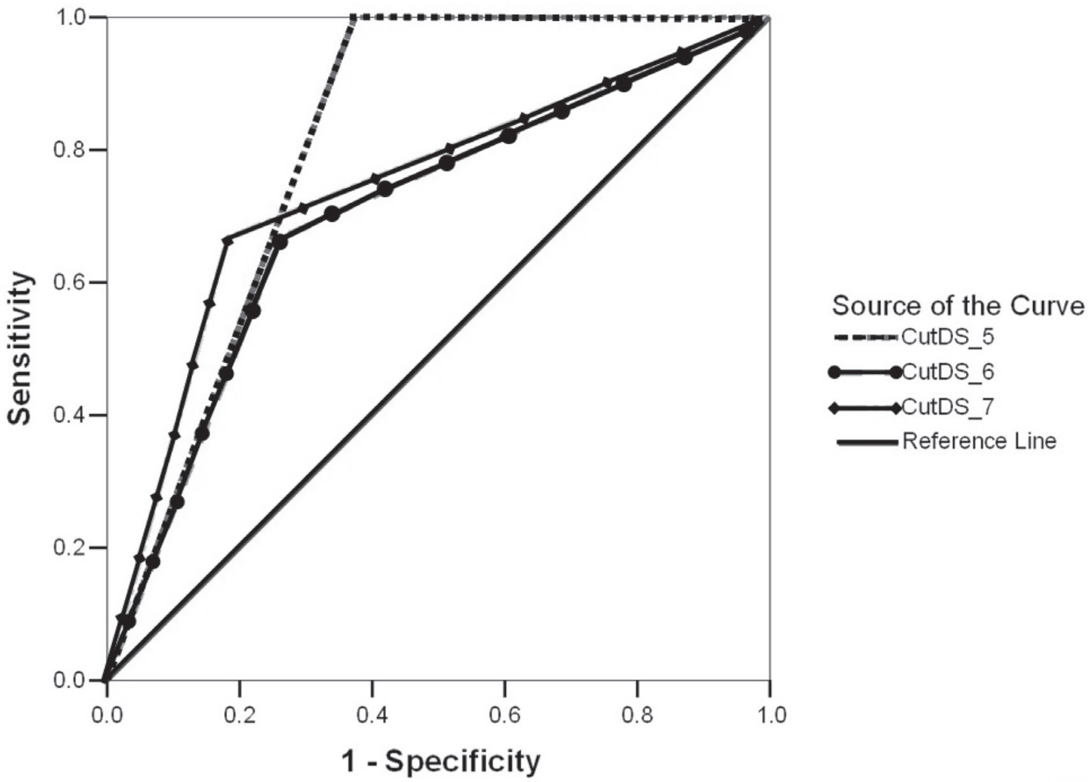


Figure 2. ROC of different cutoffs, i.e. 5, 6, 7 of dietary iron score against the probability of adequacy (PA) cutoff of 0.75; AUCs were 0.81, 0.70, 0.74, respectively

Table 4. Comparison of sensitivity and specificity of the three selected DIS cutoffs

DIS cutoff	*Sensitivity (%)*	*Specificity (%)*
5	62.9	41.7
6	75.1	41.7
7	82.9	50.0

DISCUSSION

Development of DISS

In developing a DIS, availability of quality data and accuracy of databases are critical components. The INMUCAL–Nutrient 4.1, was reviewed and iron content in foods on a dry basis were compared, e.g. cooked and raw forms of the same food item. Where there was discrepancy or unusual values, it was re-checked with another database, such as the USDA database. The scoring system developed here was based on the nutrient contents of foods which are ready to eat, not in the raw state. Therefore, for ranking the iron scores, iron content of food in the state that it is consumed (raw or cooked) was used. If the iron content of the cooked state is not available in the database, adjustment for percent loss was performed using available published information.

According to the current database (INMUCAL-Nutrient 4.1) for calculating nutrients from Thai diets (Institute of Nutrition Mahidol University, unpublished data on phytate and tannin contents were limited. Borrowing data from other sources was inevitable, and was done adjusting for moisture contents of the foods. It is also recognised that maturity of plants, length and manner of storage, and different milling fractions result in wide variations of the phytate content in foods or food products formulated from phytate-containing raw materials (Gibson & Ferguson, 1999). These factors should be considered in adjusting the final phytate values. However, only moisture content was used in adjusting for borrowed data because of limited data on other factors. Another crucial problem encountered was the use of different analytical methods to quantify phytate contents. The high-performance liquid chromatography (HPLC) technique provides more specific, sensitive and accurate phytate contents. It is the inositol phosphate, not the total phosphate, which exerts inhibitory effect on iron absorption. Unfortunately, it is not always available in food composition databases and literature. Thus, the total phytate-P value was used for this study. In this way, the modifying effect of a meal which include food items with phytate might be underestimated.

In the development of the DISS, it was first thought that summation of inhibiting and enhancing scores derived from the absorption ratios of various individual foods would be valid. However, when the MS of foods were summed for all food items contained in a meal, it was found to be impractical, since the net modifying effects in a meal is the final result of interactions between food components, which is not quantifiable from the calculation of absorption ratios of individual foods, as was obtained in estimating MS. Comparatively, the absorbable iron derived using the Hallberg & Hulthen algorithm (Hallberg & Hulthen, 2000) seems advantageous since it includes various possible key enhancers and inhibitors of iron absorption known to date. Hypothetical meals which provided the absorbable values for all possible combinations of foods in the habitual diets were formulated and the net modifying effects of all dietary modifiers contained in meals were calculated as AR. Among the hypothetical meals, the range (minimum to maximum) of vitamin C, MFP, phytate, calcium, tannin, and egg were 0-212 mg, 0-39 g, 0-114 mg, 3-289 mg, 0-158 mg, and 0-1 egg, respectively**.** These maximum amounts of inhibitors (i.e. phytate, calcium, and tannin) corresponded to almost the maximum threshold of inhibiting effect (Hallberg & Hulthen, 2000). The maximum threshold effect of vitamin C was difficult to establish since it varied according to the molar ratio of iron and concurrent phytate and tannin contents (Teucher, Olivares & Cori, 2004). However, up to 500 mg vitamin C added to the meal showed significantly increased enhancing effect (Siegenberg *et al.*, 1991). In terms of portion size, in order to make sure that the PME equation is applicable for the population, it has to be verified that the increase in size of foods composed in a meal may change the modifying effects. Thus, that the same relationship (slope and validity) holds when higher vitamin C (>212 mg) and MFP (>39 g) were consumed, e.g., in a larger consumption size of adults.

The MS was constructed based on the amounts of dietary modifiers corresponding to the consumption size. In the present study, portion size of school children was used. Hence, this modifying score may not be appropriate if it is applied to an adult's dietary intake. Verification may be needed to examine whether the final modifying scores, when they are constructed based on the adult's dietary patterns and consumption sizes, will concur with the results obtained from intakes of school children in this study.

Before further application of DISS, it is advisable that the following conditions are verified:

1) Dietary patterns of the target population: Since this DISS was derived based on specific sets of meal combinations common in Thai diets, testing DISS with other habitual dietary patterns which are both more and less monotonous should be performed.
2) Since the summed MS is dependent on portion size consumed, further investigations whether there is any threshold level when consumption size of a particular food items is larger than that used in this study should also be performed.

Validation of DISS against absorbable iron derived by the Hallberg & Hulthen algorithm

The performance of the DISS in screening populations at risk for iron inadequacy was tested with the secondary dietary data. The correlation between DIS and absorbable iron calculated by the Hallberg & Hulthen algorithm was significant (r=0.34, p<0.001) (Figure 1). It was stronger than the correlation between DDS (counting numbers of food groups consumed) and estimated absorbable iron (r=0.11) reported by Kennedy *et al.* (2007). This suggested that including the influences of dietary iron modifiers in formulating dietary score for iron may result in an improved predictive ability of simplified dietary scoring.

In establishing the cutoff for DIS to classify people at risk of inadequate iron intake, the three cutoffs were found to be reasonable according to the high AUC of ROCs. Among the cutoffs tested, 5, 6 and 7, the best sensitivity (Se) and specificity (Sp) was for cutoff 7 (Se=82.9%, Sp=50.0%) (Figure 2). The Kappa statistic, however, was rather low for all cutoffs used (Kappa = 0.06, 0.12 and 0.15 for the dietary iron cutoff of 5, 6 and 7, respectively).

Limitations of study

The present study has several limitations that must be considered in validating or improving this scoring system, specifically including several days of dietary intake data, consumption data of other age/population groups to account for wider range of iron intakes and portion size. These will need to be verified before the developed DISS can be recommended for use. In addition for testing intake adequacy using DISS, the relationship between DISS and biochemical parameters such as ferritin will be useful to strengthen the validity of this newly developed scoring system.

Interpretation of the findings include the point that the data from one day intake of most of the children (92.0%) in this study showed rather low iron intakes, and hence, high probability to have inadequacy. In fact, efforts were made in some steps, e.g. iron score, to include foods which might not be consumed by children in this data set, but which are known to be common in the habitual diets, however, there seems to be other issues that need empirical testing. Validation may be repeated with a population's consumption when the intake distribution covers a wider range of iron intake. In addition, the absorbable iron calculation was based on nutrient contents of raw foods that may result in overestimating the amount of vitamin C. Hence, the enhancing effect of vitamin C leads to overestimated absorbable iron.

CONCLUSION

To the best of our knowledge, this is the first study that takes into consideration the interactions among dietary factors affecting bioavailability of dietary iron to develop a simplified DIS. It was intended to be used as a field-friendly tool for screening populations at risk of iron inadequacy. Although more verification and validation study may still be needed, the DIS is potentially useful for monitoring iron adequacy in a programmatic context. Further simplification may still be needed to reduce the burden of calculation.

List of Abbreviations

AR: Absorption ratio; AR-Enhancer: Absorption ratio for enhancers; AR-Ca: Absorption ratio of calcium; AR-Inhibitor: Absorption ratio for inhibitors; AR-MFP: Absorption ratio for meat-fish-poultry; AR-Phyt: Absorption ratio for phytate; AR-tannin: Absorption ratio for tannin; AR-VC: Absorption ratio for vitamin C; AUC: Area under the curve; DDS: Dietary diversity score; DIS: Dietary iron score; DISS: Dietary iron scoring system; EAR: Estimated average requirement; IS: Iron score; MFP-factor: Meat fish poultry factor; MS: Modifying score; PA: Probability of adequacy; PI: Probability of inadequacy; PME: Predicted modifying effect; ROC: Receiver operating curve; Se: Sensitivity; Sp: Specificity; VitC: Vitamin C

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Authors' contribution

LC, PW, NP and EW planned the study; LC wrote the manuscript with advice and editing by PW and inputs from the other authors.

Conflict of interest

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