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Original Research

Development of a Database for the Estimation of Heme Iron and Nonheme Iron Content of Animal-Based Foods

Martha Carolina Archundia-Herrera 1 1 , Fernanda Nunes 2 2 , Isabella D Barrios 1 , Clara Y Park 3 3 , Rhonda C Bell^{[1,](#page-0-0)*}, Kimberly O O'Brien^{[2](#page-0-0),[**](#page-0-2)}

 1 Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada; 2 Division of Nutritional Sciences, Cornell University, Ithaca, NY, United States; ³ Division of Food and Nutrition, Chonnam National University, Gwangju, Republic of Korea

ABSTRACT

Background: Total iron (TI) intake and differentiation between heme iron (HI) and nonheme iron (NHI) are uncommon despite markedly different bioavailability.

Objectives: To create a database compiling information from studies that directly assessed the HI content of animal products using the Hornsey method, and to explore differences in estimates of HI intake between the data compiled and the Monsen method.

Methods: A literature search identified studies that chemically characterized the HI content of animal-based foods using the Hornsey method; HI, NHI, and TI contents (mg/100 g) were compiled. Information was grouped by animal type and cooking method, and mean (SD) HI% was calculated. Using a 24-h dietary record, differences in HI and NHI intake using the compiled information and the Monsen approach were explored.

Results: Actual HI% values ranged from 7% to 94%. Raw foods had the highest HI% [raw duck (94% \pm 4%), raw blood curd (82% \pm 4%), and raw beef (79% \pm 9%)]. Boiled foods had the lowest HI% [boiled shrimp (11% \pm 5%) and meatballs (15% \pm 6%)]. Cooked foods with the highest HI% were beef (70% \pm 10%) and lamb (70% \pm 9%). In many instances, applying actual HI% from the complied database produced markedly different measures of the HI content of foods [cooked beef (Monsen: 1.3 mg/100 g); (Hornsey: 2.3 mg/100 g)]. Estimation of iron intake in a 24-h recall demonstrated that using animal-specific HI% results in different estimates of HI intake [Monsen: 1.2 mg HI (40%); Hornsey: 1.8 mg HI (59%)].

Conclusions: Animal-based foods have variable HI%. A fixed HI:NHI ratio does not reflect this variation and could give rise to inaccurate estimates of HI content in food and HI intake. Consideration of this variation in HI% may improve our ability to link dietary intake with iron status and important health outcomes.

Keywords: heme iron, nonheme iron, total iron, Hornsey method, Monsen method

Introduction

Although iron is the fourth most abundant mineral on earth, iron deficiency remains a significant public health problem. Iron is found in the foods we eat, mainly as nonheme iron (NHI) in the form of ferric iron (Fe^{3+}). Absorption of NHI is influenced by dietary enhancers and inhibitors and by the iron status of the host, as mediated by the iron regulatory hormone hepcidin. Heme iron (HI) is found in animal-based foods including meat,

poultry, and fish. Absorption of HI is mediated by an entirely different pathway that is not substantially impacted by iron status, dietary inhibitors or enhancers, or systemic hepcidin concentration [\[1](#page-6-0)]. Although humans only ingest 10%–15% of their total iron (TI) intake as HI, it is estimated to account for \geq 40% of the TI absorbed [[2\]](#page-6-1). Thus, iron bioavailability depends on the relative amounts of HI and NHI in the diet [[3\]](#page-6-2).

Although the bioavailability of HI and NHI is markedly different, in general, studies of iron intake in humans report only

Abbreviations: CNF, Canadian Nutrient File; HI, heme iron; NHI, nonheme iron; TI, total iron.

^{*} Corresponding author.

^{**} Corresponding author. E-mail addresses: rhonda.bell@ualberta.ca (R.C. Bell), koo4@cornell.edu (K.O. O'Brien).

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TI intake and rarely differentiate between the sources of iron as HI and NHI. Nutrient composition databases, including the Canadian Nutrient File (CNF) [[4\]](#page-6-3) and the USDA Food and Nutrient Database for Dietary Studies [[5\]](#page-6-4), also do not report the amounts of HI and NHI in foods.

To estimate the HI and NHI content of animal products, Monsen et al. [[6\]](#page-6-5) proposed that 40% of TI was HI in all animal-based foods, based on the premise that the proportion of HI in animal-based foods varies little among types of animal meats. However, this assumption is not supported by the literature, as chemical analyses of the HI content of animal-based foods suggest that it varies considerably [\[7](#page-6-6),[8\]](#page-6-7). Several chemical approaches have been used to directly measure the HI content of individual animal products. The Hornsey method [\[9\]](#page-6-8) is the most widely applied method to chemically assess the HI content of animal-based foods [\[7](#page-6-6),[10](#page-6-9)–[14](#page-6-9)]. This method directly measures the amount of iron from extracted heme pigments [\[9](#page-6-8), [15\]](#page-6-10). Determinations of HI content of animal-based foods using this method note that HI can range between 22% and 80% of the TI content of different animal-based foods [[7](#page-6-6)[,8](#page-6-7)].

Although several investigators have reported the proportions of HI and NHI, this information is not easily accessible and thus cannot be readily applied to dietary iron intake data. Creating a database that contains detailed information on the HI content of animal products may improve our understanding of the contribution that HI and NHI play in affecting iron bioavailability and iron homeostasis. To address this gap, the aim of this study was to search the literature for studies that determined the HI content of animal-based foods using the Hornsey method and to create a database that compiled the information for HI, NHI, and TI content of these products. Animal-based foods were categorized by animal type (beef, pork, lamb, etc.), meat cut (loin, fillet, breast, etc.), and cooking method (raw, cooked, and boiled) and mean \pm SD values for HI% and NHI% were calculated using the compiled database. These values were compared with the 40%:60% fixed ratio suggested for HI%:NHI% values using the Monsen approach. The 2 approaches were applied to a sample 24-h dietary recall to understand the impact of using each approach to estimate HI and NHI intake.

Methods

Literature search

A search of the available literature that measured the HI content of animal products using the Hornsey method was undertaken. PubMed and Web of Science were searched for English-language articles using the following search strategy. PubMed: (heme[mesh] OR Haem[tiab] OR heme[tiab]) AND (meat[tiab] OR poultry[tiab] OR fish[tiab] OR chicken[tiab] OR turkey[tiab] OR beef[tiab] OR lamb[tiab] OR pork[tiab] OR shellfish[tiab] OR food*[tiab]) AND (content*[tiab] OR composition[tiab]); Web of Science: TS=((heme OR haem) AND (meat OR poultry OR fish OR chicken OR turkey OR beef OR lamb OR pork OR shellfish OR food*) AND (content* OR composition)).

Studies were included if they: 1) assessed HI, NHI, TI, or HI% contents of animal-based foods, 2) used the Hornsey method to quantify the HI content of this food, because different biochemical methods can yield statistically different results [\[7\]](#page-6-6), 3) reported results in tables, paragraphs, and/or graphs with

clearly labeled axes, and 4) presented HI as mass per mass of TI (for example, μ g/g, mg/100 g, etc.) or as a percentage (% of TI). Studies were excluded if they used any other analytical method to quantify the HI content of the animal-based food.

Data extraction

Each study was reviewed, and the data were extracted by 2 independent authors (FN and IDS) and checked for consistency by a third author (MCAH). The information extracted included: the animal source of the food, the condition of the animal product at the time it was analyzed (for example, dry or fresh weight), the cooking method used prior to assessing iron content of the product, and any of the following that were available: HI content (mg/100 g), NHI content (mg/100 g), TI content (mg/ 100 g), and HI proportion $(\%)$.

Development of database

Once the information was extracted, values for HI, NHI, and TI content, along with the proportion of HI (%), were organized in an Excel spreadsheet by categories of cooking methods and animal-based foods (beef, lamb, pork, chicken, turkey, etc.). Within each species, categories were created for different cooking methods (raw, cooked, and boiled/steamed). Cooking methods were separated because they can influence the iron content of foods [[16\]](#page-6-11). In cases where NHI was missing from a study, it was calculated as follows: NHI (mg/100 g) = TI $(mg/100 g) - HI (mg/100 g)$. HI% was calculated for all studies as [HI (mg/100 g) \times 100)]/TI (mg/100 g). Next, mean values (\pm SD) for HI (mg/100 g), NHI (mg/100 g), TI (mg/100 g), and HI% were calculated for each animal-based product using all values within that animal group and cooking method category.

For ease of description, we classified beef, pork, lamb, and horse as "red meats," chicken, turkey, duck, rabbit, ostrich, and rhea as "white meats," and all fish and shellfish as "seafood." Sausage and meatballs were a discrete group because of the high likelihood that they contain additional ingredients that could reduce the concentration. Liver and blood curd were also a discrete group because their iron content is higher than many other animal-based foods.

Comparison of HI content of animal products calculated using HI proportions from the compiled database and the Monsen approach

The TI content of commonly consumed animal products (beef, pork, lamb, chicken, turkey, etc.) was extracted from the USDA National Nutrient Database for Standard Reference, Legacy [\[5\]](#page-6-4). HI content of these products was calculated by applying the HI% values compiled in the new database and those obtained by applying the fixed fraction of 40% HI and 60% NHI recommended by the Monsen method.

Differences in dietary HI intake from a sample 24-h dietary recall calculated by using HI proportions determined in the compiled database and the Monsen approach

The differences in the estimates of HI and NHI intake obtained using these 2 approaches were examined as a first step to understand the importance of applying the varying HI% values from the compiled database and the fixed fraction (40% HI and 60%

FIGURE 1. Flowchart of literature review of heme iron content of animal-based foods.

NHI) in dietary assessment. A 24-h dietary recall example was randomly selected from an extant database, and the TI contents of each food were recorded from the USDA database. The TI (mg) for the animal-based foods consumed was multiplied by the HI% derived from the compiled database and by the fixed fraction of 40% for HI proposed by Monsen [[6\]](#page-6-5). TI, HI, and NHI intakes (mg) were calculated by summing across all iron-containing foods.

Results

The literature search yielded 947 articles, after removing 254 duplicates, a total of 693 articles were screened for eligibility ([Figure 1\)](#page-2-0).

Of the studies identified, 660 were excluded because they did not pertain to the topic of interest. The full text of 36 studies was reviewed to further assess eligibility. Of the 33 studies using the Hornsey method, an additional 3 articles were added by crossreferencing, and 12 did not meet all eligibility criteria, leaving 24 studies to be used for creating the database. Supplemental Material 1 contains general information about these studies and the methods used to determine HI, NHI, and TI. A total of 279 HI values were extracted from the eligible studies (Supplemental Material 2). Missing information was noted. The HI, NHI, and TI contents and the proportions of HI and NHI of the animal-based foods by species and cooking status are presented in [Table 1](#page-3-0).

Supplemental Material 3 shows the mean values for each animal species by cooking method and cut. Of the 279 values extracted, 15 values (6%) were >2 SDs below or above the mean of their respective category, thus, they were considered outliers and were not included in calculations of the means (Supplemental Material 4).

Raw animal-based foods with the highest HI% were raw duck (94% \pm 4%), raw blood curd (82% \pm 4%), and raw beef and rhea (79% \pm 9%), whereas HI% was lowest in boiled animal-based foods. The products with the lowest HI% were boiled shrimp (11% \pm 5%) and boiled meatballs (15% \pm 6%). Cooked food products had lower HI% than most raw foods. For example, the HI% content of cooked red meats was \sim 10%–13% lower than raw red meats (13% lower in beef, 11% lower in lamb, and 10% lower in pork). The HI% in cooked white meats was lower than that of raw white meats, and the difference was larger than that between cooked and raw red meats. For example, the HI% of cooked chicken was \sim 50% lower than raw chicken, and 27% lower in cooked compared with raw turkey. The HI% in cooked fish was 34% lower than the raw samples. Cooked sausages were 18% lower in HI than the raw product, liver HI% was 36% lower in cooked liver than raw liver, and blood curd was 9% lower in cooked blood than unprocessed blood. In contrast, the HI% was 39% higher in steamed compared with raw clams.

Heme, nonheme, and total iron content (mg/100 g) and proportions (%) of heme iron and nonheme iron, categorized by animal-based food and cooking statu $\bar{\mathbf{s}}$

Percentages of HI and NHI based on calculations in the compiled database of samples analyzed using the Hornsey method

From the compiled data, measuring HI using the Hornsey method resulted in a wide range of HI% from 7% to 94% in different animal-based foods. Raw beef, lamb, pork, chicken, turkey, duck, rabbit, rhea, fish, and blood curd all had a HI proportion above 40%, with values ranging from 50% to 94% ([Figure 2](#page-4-0)A). Proportions of HI and NHI in raw green mussels and prawns were similar between the 2 methods, whereas the HI% was <40% for uncooked clams, sausages, meatballs, and liver. Similarly, using the HI% values calculated in the complied data, cooked beef, pork, lamb, horse, rabbit, and ostrich had HI% above 40% (range: 52%–75%), cooked cockles had 40% HI, and cooked chicken, turkey, fish, sausage, and liver have a HI% below 40% (range: 33%–36%) [\(Figure 2](#page-4-0)B). Many boiled animal products had a HI% <40% of TI including boiled fish, clams, green mussels, prawn, sausage, meatballs, and liver (range: 11%–27%), although some remained above 40%; boiled pork was the meat closest to 40%, the value proposed by Monsen et al. [\[6](#page-6-5)] ([Figure 2](#page-4-0)C).

Estimates of HI and NHI content in animal-based foods using proportions calculated in the compiled database and the Monsen approach

Values for HI% and NHI%, calculated from the compiled database and the Monsen approach were used to estimate the HI and NHI content (mg/100 g food) of popular animal-based foods ([Table 2](#page-4-1)).

In general, if the TI reported in an animal-based food is high, even a small difference in percentage may result in a large absolute difference in HI. If the TI reported in an animal-based food is low, even if the HI% differs a lot, the absolute difference would be small. For example, beef and lamb have high TI and HI%, so the difference between methods is big; the Monsen approach resulted in a 65% lower estimate of HI content by the Monsen approach in cooked beef compared with the newly compiled database (Monsen: 1.3 mg/100 g; Hornsey: 2.3 mg/100 g). Chicken, turkey, and fish have similar HI%, so they do not differ drastically. Both meatballs and shrimp have much lower HI% in this database than in the Monsen method, but meatballs have a higher TI, so the absolute difference between the 2 methods is greater in meatballs than shrimp.

Differences in estimates of HI and NHI intake for a 24-h recall using proportions of HI and NHI calculated from the compiled database and the Monsen approach

An example of applying the 2 approaches to estimate HI and NHI intake in a 24-h recall is presented in [Table 3](#page-5-0). The TI value of all the food items consumed was 11.1 mg. The TI from animalbased foods was 3.0 mg equivalent to 28% of the iron consumed that day. According to the Monsen approach, 1.2 mg (40%) is HI and 1.8 mg (60%) is NHI. In contrast, applying the HI % calculated from the compiled database indicates 1.8 mg (59%) is HI and 1.3 mg (41%) is NHI.

Discussion

This project compiled a database of the HI and NHI content of different animal products using published data that determined

 $\overline{ }$ Steamed.

 \mathbf{I}

The Common

Animal species

FIGURE 2. Comparison of the proportions of HI and NHI in animal-based foods using the compiled database. The Monsen approach is provided for comparison as the red line. (A) HI% and NHI% content in raw animal-based foods, (B) HI% and NHI% content in cooked animal-based foods, and (C) HI% and NHI% content in boiled animal-based foods. HI, heme iron; NHI, nonheme iron.

TABLE 2 Estimates of HI and NHI content of animal-based foods using proportions calculated from the compiled database and the Monsen approach

Food description information was used as published, with no edits or transformations from USDA National Nutrient Database for Standard Reference, Legacy.

Abbreviations: HI, heme iron; NHI, nonheme iron.

HI by chemical analysis using the method developed by Hornsey et al. [\[6](#page-6-5)]. The proportions of HI and NHI, relative to TI, were calculated in each animal product and then categorized based on animal species and cooking status and methods. We observed that the HI% in animal-based foods ranged widely, from 7% to 94% of TI, suggesting that using a fixed fraction of 40%:60% for HI:NHI may not be appropriate for estimating the HI and NHI content of animal-based foods and may lead to inaccurate estimates of HI and NHI intakes. The estimates of HI and NHI intakes calculated in the sample 24-h recall used the 2 different approaches (that is, applying the HI% and NHI% from the compiled database and using a fixed fraction of 40%:60%, HI%:NHI%) highlight the variation between these approaches. The impact of this variation is currently unclear; however, the results may be

useful to more fully understand the bioavailability of iron and the relationship between iron intake, from foods and supplements, and markers of iron status. These important studies should be a focus of future research.

Humans maintain iron homeostasis by absorbing HI and NHI from a range of dietary sources. HI and NHI vary considerably in terms of the amounts absorbed, the mechanisms of absorption, and the degree to which regulatory hormones and dietary inhibitors and enhancers affect absorption and status. Better quantification of the form of iron ingested may advance our understanding of the relationship between dietary iron intake and markers of iron status. Systematic reviews have shown that nonvegetarians have higher serum ferritin levels than vegetarians, even with lower TI intakes [\[17](#page-6-12),[18\]](#page-6-13). This could be an

TABLE 3

Example of differences in estimates of HI and NHI content for a 24-h recall using proportions calculated from the compiled database and the Monsen approach

Abbreviations: HI, heme iron; NHI, nonheme iron.

outcome of higher bioavailability of HI. Young et al. [\[1\]](#page-6-0) noted that HI intake from animal-based foods is an independent predictor of serum ferritin in healthy young females, supportive of the variable bioavailability between HI and NHI. Future studies that estimate HI and NHI content in foods using the refined methods proposed here may better capture relationships between the HI and NHI dietary contents and diet-based study outcomes. Together, these insights may help to propel this field forward.

The newly complied HI database highlights the impact of cooking status (raw or cooked) and cooking methods that may merit more attention when estimating dietary iron intake. For example, cooked animal products generally have higher amounts of TI (per 100 g sample) than their raw counterparts because of losses in sample mass (for example, fat is lost during cooking). Cooking at temperatures above 85° C results in iron being released from its heme complex, causing oxidative cleavage of the porphyrin ring [[19\]](#page-6-14) and losses in HI%. Gandemer et al. [\[20\]](#page-6-15), modeled the kinetic effects of cooking mode, time, and temperature on the HI content of meat [\[20](#page-6-15)]. Longer cooking times and higher cooking temperatures resulted in a greater loss of HI. Losses also occurred because of juice expulsion and by the conversion of HI into NHI [[20\]](#page-6-15). Boiling may facilitate the loss of iron-containing juices into the surrounding liquid, thereby lowering the HI content of an animal product. Additional research in food science could refine our understanding of the factors that affect the HI content and HI% of foods. Furthermore, it highlights the importance of considering both the HI proportion and the TI content (mg/100 g) of specific foods when identifying foods that

are considered "high in iron." For instance, cooked beef liver has a TI content of 6.54 mg/100 g, is considered "high in iron," and 33% of the TI is HI. Interestingly, in chicken, 33% of the TI is HI. However, the HI content of liver is 2.15 mg/100 g, whereas the HI content of chicken is 0.16 mg/100 g.

Current health recommendations promote reduced consumption of animal-based foods and increased intake of plant-based foods. Indeed, many plant-based foods are marketed as replacements for animal-based foods [[21\]](#page-6-16). Although the movement to increase plant-based foods in the diet can contribute to a reduced risk of noncommunicable diseases, the impact of this substitution on micronutrient intake and status requires further investigation. Moving to include food-specific information about HI and NHI contents and proportions in food composition tables is not an easy task, although the development and availability of the newly compiled data is an important step in this direction. Additional work is needed to broaden the numbers and types of foods with more complete HI and NHI information. The iron content of foods may also change over time, including animal-based foods [\[22](#page-6-17)]. This work should be undertaken in collaboration with food scientists using up-to-date approaches as well as regulatory groups that are part of the food composition tables.

The strengths of this study include the compilation of chemically analyzed HI content in animal products using the Hornsey method in an accessible and publicly available database. Some potential limitations of this approach are that although we classified values by animal species, additional factors may influence HI content or proportions within a species, including genetics, slaughter practices, type of animal feed, preparation and storage of samples, seasonal variations, and geographic location [[23\]](#page-6-18). The shellfish group was particularly challenging to describe because the HI% varied considerably between different species in this group. For example, the variation in the HI% content of clams may be the result of environmental conditions and processing methods; the presence of grit was positively correlated with higher iron content [[24\]](#page-6-19). More analyses using the Hornsey method using different types of shellfish could help improve the information available on the HI, NHI, and TI content of these items.

In conclusion, this database provides researchers with open access to a compilation of studies on chemically analyzed HI% of animal-based products. The current database strongly indicates that HI and NHI values should not be assumed to be a constant percentage of TI, but should be specific for each animal-based food type. Hence, a more accurate estimation of iron intake should consider the specific animal-based food type consumed and the cooking method used to provide a more reliable estimate of iron intake. This database may foster our understanding of HI and NHI consumption, which may contribute to a better understanding of the relationships between dietary iron intake and relevant health outcomes.

Author contributions

The authors' responsibilities were as follows – RB, KO, CYP, MCHA: designed research; FN, IB, MCAH: conducted research; MCHA, RB: analyzed data; MCAH, CYP, KO, RB: wrote the article; RB: KO: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript that were used to compile the HI, HI%, NHI, NHI%, and TI of the animal products, including references to the original citations, will be made publicly and freely available without restriction.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cdnut.2024.102130>.

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