

Total Iron and Heme Iron Content and their Distribution in Beef Meat and Viscera

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Received: 28 April 2009 / Accepted: 30 April 2009 /
Published online: 28 May 2009
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Abstract To determine the content of total iron (TFe) and heme iron (HeFe) in major cuts of meat and principal viscera of bovine origin. ^{55}Fe (30 mCi) was injected into two 4-month-old calves. Triplicate samples of the 12 basic American cuts of meat and major viscera were obtained from each specimen. Samples were acid digested and their iron content was read by atomic absorption spectrophotometry. Duplicate samples of the basic cuts of meat and major viscera were analyzed to determine the concentration of ^{55}Fe using a double isotopic technique. The mean and standard deviation of TFe for all cuts was 1.4 ± 0.3 mg/100 g of meat. The mean TFe for organs was (per mg/100 g): 0.9 ± 0.1 brain, 3.0 ± 0.05 kidney, 3.2 ± 0.04 heart, 5.7 ± 0.2 lung, 6.0 ± 0.1 liver, and 31.2 ± 0.4 spleen. HeFe was 64% of TFe in meat and 72.8% in spleen, 53.8% in lung, 35.7% in brain, 35.0% in kidney, 27.3% in heart, and only 13.6% in liver. Blood contained 85.5% of the radioisotope and only 1.4% was found in muscle and 1.6% was found in viscera. Results suggest that bovine cuts of meat have a low variation in TFe and that HeFe comprises more than 60% of TFe.

Keywords Iron · Heme iron · Meat · Viscera · Radioisotopes

Abbreviations

TFe Total iron
HeFe Heme iron
nHeFe Non-heme iron

Introduction

The diet of populations in developing countries is usually low in micronutrients, which is especially true for bioavailable iron [1]. Low intake of bioavailable iron can lead to iron

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deficiency anemia, particularly among children, adolescents, and women of child-bearing age [2–5]. Iron deficiency has significant consequences for human health in general and child development in particular, and in those countries most affected by the condition, it can result in a negative economic impact. For this reason, efforts to improve the nutritional status of this micronutrient on a worldwide level are a priority in the field of public health [6].

Iron, which is found in a large variety of foods, can be found in the diet in two forms, each having different routes of intestinal absorption. Non-hemic iron (non-heme iron or inorganic iron) is present in vegetables and dairy-based foods, while hemic iron (heme iron) is present in foods derived from animal tissue. One of the most iron-rich food sources is bovine meat (beef). Iron in beef meat exists as both heme iron (HeFe), as a component of myoglobin in the muscle, and as non-heme Fe (nHeFe) [7]. Nevertheless, their bioavailability differs; HeFe being highly bioavailable and significantly more so than nHeFe [8–11]. HeFes has high bioavailability given that it has few factors that interfere with its absorption in the intestinal lumen and because HeFe is absorbed by the enterocyte as an intact molecule [12–14].

To quantify adequate iron consumption at the population level, food composition tables with chemical content are needed. However, the content of iron has been determined for only a few cuts of meat and there is no information on the content of HeFe or on the heme/non-heme iron ratio in these tables. Therefore, the objective of the present study was to determine the amount of total Fe (TFe) and HeFe in different cuts of American beef and in the principal bovine organs via the administration of a radioactive dose of ^{55}Fe and the determination of its distribution within the aforementioned components.

Materials and Methods

Animals

The protocol of the study was approved by the Bioethics Committee for Experimentation with Animals of the Institute of Nutrition and Food Technology (INTA). Two male Holstein–Friesian bovines, 4 months of age, were utilized. The calves were raised for a period of 2 months. At the beginning of the period, a disease control exam was conducted, which included tests for parasitic hematophages and the tuberculin test. The animals, which were maintained in a corral physically adapted in accordance with the Manual of Biosecurity Standards of the Chilean Council of Science and Technology [15], were fed daily 3.2 kg of alfalfa hay and 1.5 kg of concentrate for beef cattle (Champion, Santiago, Chile). Water was provided *ad libitum*. The total iron content of the alfalfa hay and concentrate was assessed from random samples, by wet acid digestion [16], and subsequent analysis in an atomic absorption spectrophotometer (Perkin-Elmer 2800). Results showed that the food provided satisfied the iron requirements of the calves.

Radioisotope Administration and Iron Nutritional Status

A single dose of 20 mCi of the iron radioisotope ^{55}Fe (NEN, Life Science Products, Inc., Boston, MA, USA) was injected into the jugular vein of each animal after nocturnal fasting at the beginning of the 2-month period. Every 15 days, a sample of blood (6 ml) was taken from the jugular vein in order to monitor the incorporation of ^{55}Fe in the blood and to assess hemoglobin concentration (Hb), hematocrit (Hct), median corpuscular volume (MCV), and zinc protoporphyrin concentration (ZPP). Hb, Hct, and MCV were determined

using an electronic cellular counter (CELL-DYN 1700 Diagnostics, Abbott Park, IL, USA) and ZPP was assessed using a hematofluorometer (AVIV Biomedical Inc., Lakewood, NJ, USA model 206D).

Sampling of Meat and Viscera

The calves were slaughtered in the Animal Necropsy Laboratory of the Faculty of Veterinary Sciences, University of Chile. They were first anesthetized (10% thiopental at 20 mg/kg administered by I.V.) and later sacrificed by exsanguination. A professional butcher identified and dissected, as specified by American standards [17–19], the 12 cuts of meat from each hemicanal of each animal. In addition, the heart, lungs, spleen, liver, kidneys, and brain were extracted. The cuts and viscera were individually weighed and classified. Given that the weight and number of muscles differ between the different cuts of meat, a sampling number was ascertained for each cut based on the proportional weight of the sample and on the number of muscles forming each cut. Samples from each cut were randomly selected equidistant from each other and dissected into 0.5-cm-thick segments, each weighing 20 g. Individual samples were stored in sealed polyethylene bags which were coded in accordance with the identification number of each animal and the hemicanal to which it belonged. All samples were maintained at -20°C until the time of their analysis and later processed in triplicate. Table 1 shows a list of the 12 cuts of meat obtained along with the muscles of which each cut is composed. Figure 1 displays a diagram of the 12 beef cuts according to American standards [19].

Prior to preparation, the samples were defrosted and the extramuscular fat removed so that the analysis would be based on lean meat. TFe was determined using atomic absorption spectrophotometry (AAS) after acid digestion [16]. nHeFe content was also determined by AAS, but after acid extraction [20]. HeFe content was calculated from the difference of TFe and nHeFe. Results are expressed in mg/100 g.

Distribution of ^{55}Fe Dose in Muscles and Viscera

The ^{55}Fe radioisotope content in the meat and viscera was determined using a double isotope technique [21]. The quantification of ^{55}Fe was conducted using a liquid scintillation counter (Packard Canberra Company TRI-CAB 1600 TR). The results were expressed as cpm/g and as a percentage of the total administered labeled dose.

Statistical Analysis

Descriptive statistics were calculated for each of the variables. A Pearson r test was used to determine correlations in both TFe and HeFe content between the two animals and between the right and left hemicanals of each animal and to determine correlations between TFe and HeFe content in the meat and viscera (Statistica for Windows 5.1 1997, Statsoft Inc., Tulsa, OK, USA).

Results

Results of the hematological parameters showed that both calves had normal iron status [22]. The mean and standard deviation of Hb, Hct, MCV, and ZPP were 117 ± 11 g/l, $19.4 \pm 3.9\%$, 33.2 ± 1.5 fl, and 1.16 ± 0.28 $\mu\text{mol/l}$, respectively.

Table 1 Scientific Names of Muscles that Compose each of the 12 Cuts of American Beef

American cut	Muscles (scientific names)
Neck	<i>M. trapezius pars cervicalis</i> , <i>M. brachiocephalicus</i> , <i>M. sternomastoideus</i> , <i>M. sternomandibularis</i> , <i>M. sternohyoideus</i> , <i>M. sternothyroideus</i> , <i>M. longus colli</i> , <i>M. Rectus capitis ventralis major</i>
Chuck	<i>M. subscapularis</i> , <i>M. teres major</i> , cranial dorsal portion of <i>M. serratus cervicis ventrali</i> , <i>M. omotransversarius</i> , <i>M. splenius pars cervicis cranialis</i> , <i>M. longissimus capitis et atlantis</i> , <i>M. semispinalis capitis</i> , <i>Mm. spinalis et semispinalis cervicis</i> , <i>M. longissimus cervicis</i> , <i>M. scalenus ventralis</i> , <i>M. omohyoideus</i> , <i>Mm. multifidi cervicis</i> , <i>Mm. intertransversarii cervicis</i> , <i>M. supraspinatus</i> , <i>M. infraspinatus</i> , <i>M. deltoideus</i> , <i>M. teres minor</i> , <i>M. coracobrachialis</i> , <i>M. tensor fasciae antebrachii</i> , <i>M. anconeus</i> , <i>M. pronator teres</i>
Brisket	<i>M. biceps brachii</i> , <i>Mm. pectorales superficiales</i> , <i>pectorales descendens</i> , <i>pectorales transversus</i> , <i>pectorales profundus</i> and <i>pectorales ascendens</i> , <i>M. subclavius</i> , <i>M. rectus thoracis</i>
Foreshank	<i>Mm. antebrachialis craneales</i> and <i>caudales</i> of the hand
Hindshank	<i>Mm. cranialis</i> and <i>caudalis</i> of the leg
Ribs	<i>M. transversus thoracis</i> , parts of <i>M. obliquus externus abdominis</i> , <i>M. transversus abdominis y M. rectus abdominis</i> , <i>M. serratus ventralis thoracis</i> , <i>Mm. intercostales externi e interni y, M. longus colli pars thoracicae</i> , <i>M. serratus ventralis cervicis pars dorsalis</i> , <i>M. scalenus dorsalis</i> , <i>M. serratus dorsalis cranialis</i> , <i>M. rhomboideus pars cervicale</i>
Plate	<i>M. Cutaneus</i> , <i>Diaphragma pars costales</i> , <i>Centrum tendineum et pars sternalis</i> , <i>M. latissimus dorsi</i> and <i>M. trapezius pars thoracis</i> , <i>Pars lumbalis</i> (crura of the diaphragm)
Flank	<i>M. obliquus externus e internus abdominis</i> , <i>M. rectus abdominis pars craneales</i> , <i>M. serratus dorsalis caudalis</i> , <i>M. latissimus dorsi</i> , <i>M. transversus abdominis</i>
Loin	<i>M. iliocostalis lumborum</i> , <i>M. longissimus lumborum</i> , <i>M. retractor costae</i> , <i>Mm. Rotator costae</i> , <i>Mm. Intertransversarii</i> , <i>Mm. Transversus spinalis</i> , <i>M. iliocostalis thoracis</i> , <i>M. spinalis</i> , <i>M. semispinalis thoracis</i> , <i>Mm. levatores costarum</i> , <i>Mm. multifidi thoracis</i> , <i>Mm. gluteus medius, profundus and accesorius</i>
Tenderloin	<i>M. psoas major</i> and <i>minor</i> , <i>M. iliacus</i> , <i>M. quadratus lumborum</i>
Round	<i>M. triceps surae</i> , <i>M. gluteo biceps</i> , <i>M. semitendinosus</i> , <i>M. gracillis</i> , <i>M. semimembranosus</i> , <i>M. sartorius</i> , <i>M. pactineus</i> , <i>M. adductor</i> , <i>M. obturatorius externus</i> , <i>Mm. gemelli</i> , <i>M. quadratus femoris</i> , <i>M. quadriceps femoris</i> , <i>Mm. vastus laterales, mediales e intermedius</i> , <i>M. recto femoris</i> , <i>M. tensor fasciae latae</i>
Tail	<i>M. sacrocaudalis dorsalis et ventralis medialis</i> , <i>M. intertransversarii caudae</i> , <i>M. coccygeus</i>

The mean TFe and HeFe content of the hemicanals within each calf were significantly correlated ($r=0.92$, $r=0.94$ for TFe and $r=0.97$, $r=0.86$ for HeFe). Similarly, the mean TFe and HeFe content of each animal were also significantly correlated ($r=0.84$ and $r=0.97$). The TFe and HeFe content in the 12 cuts of meat and the distribution of the radioisotopic label (percentage of the ^{55}Fe dose found in each compartment) are shown in Table 2. TFe content in the bovine cuts ranged between 1.0 ± 0.2 mg/100 g in hindshank and 2.0 ± 1.0 mg/100 g in plate. The adjusted mean of TFe for the 12 cuts of beef meat was 1.3 mg/100 g. Similarly, HeFe content in beef ranged between 0.6 ± 0.1 mg HeFe/100 g in hindshank and 1.5 ± 0.8 mg HeFe/100 g in plate. The adjusted mean of HeFe and nHeFe beef cuts was 0.86 and 0.46 mg/100 g, respectively. Thus, HeFe comprised approximately 65% of total Fe.

The TFe and HeFe content and the distribution of the radioisotopic label in major organs are shown in Table 3. TFe in the major organs ranged between 0.9 ± 0.1 mg/100 g in brain and 31.2 ± 0.5 mg/100 g, while HeFe content was between 0.3 ± 0.2 and 22.67 ± 45 mg/100 g. The spleen was found to have much higher HeFe content per gram when compared

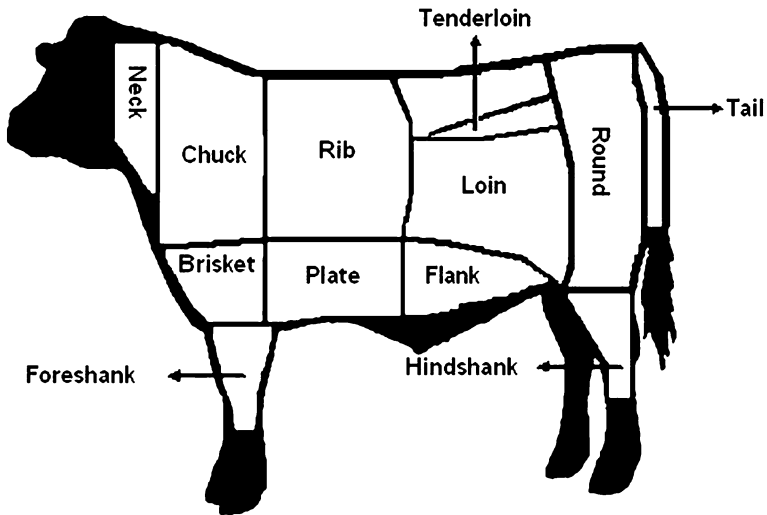


Fig. 1 Diagram of American beef cuts (standing animal). NCBA. Muscle profiling. Natl. Cattleman's Beef Assoc., 2000. Denver, CO

to the other organs, having seven times the HeFe content than the next highest organ, the lung. It should be noted that, although the liver had very low concentrations of HeFe, it was also the organ with the highest TFe content and the highest percentage of ^{55}Fe in the viscera. Expressed as percentages, HeFe comprised 73% of the iron in the spleen and more than 50% of the iron in the lung. Conversely, HeFe comprised only 14% of the TFe content in the liver.

Of the total radioactive dose of ^{55}Fe injected into the animals, only a small part reached the muscular compartment (1.4%) and the viscera (1.6%). The blood retained the majority of the labeled dose (85.5%), where it was incorporated into the red blood cells. The cuts of

Table 2 Total (TFe) and Heme Iron (HeFe) Content (mg/100 g) and Percentage of Radioisotope ^{55}Fe in Cuts of American Beef^a

American cuts	<i>n</i>	TFe	HeFe	^{55}Fe
Neck	12	1.4±0.2 (13.8)	0.9±0.1 (13.1)	2.9
Chuck	60	1.4±0.3 (19.6)	0.8±0.2 (21.9)	17.7
Brisket	24	1.2±0.2 (19.8)	0.8±0.1 (8.3)	8.3
Foreshank	12	1.4±0.3 (19.3)	0.9±0.1 (12.4)	2.2
Hindshank	12	1.0±0.2 (22.7)	0.6±0.1 (12.8)	1.8
Ribs	48	1.3±0.2 (14.2)	0.9±0.2 (18.7)	18.6
Plate	48	2.0±1.0 (50.3)	1.5±0.8 (58.0)	6.0
Flank	18	1.4±0.2 (10.9)	0.9±0.1 (14.1)	4.8
Loin	36	1.3±0.3 (22.4)	0.9±0.3 (31.5)	11.3
Tenderloin	12	1.4±0.1 (9.1)	1.0±0.1 (11.1)	2.7
Round	72	1.2±0.2 (16.7)	0.8±0.1 (17.1)	21.9
Tail	6	1.5±0.3 (20.7)	1.0±0.2 (16.3)	0.8

^aTFe and HeFe are expressed as mean±SD (CV)

Table 3 Total (TFe) and Heme Iron (HeFe) Content (mg/100 g) and Percentage of Radioactive Isotope ^{55}Fe in Major Bovine Organs^a

Organ	<i>n</i>	TFe	HeFe	^{55}Fe (%)
Spleen	6	31.2±0.4 (4.6)	22.7±0.5 (8.1)	19.6
Brain	6	0.9±0.1 (8.9)	0.3±0.2 (28.5)	0.4
Heart	6	3.2±0.04 (1.4)	0.9±0.08 (7.4)	1.9
Liver	6	6.0±0.05 (0.8)	0.8±0.09 (13.2)	56.6
Lung	6	5.7±0.2 (3.3)	3.1±0.2 (9.9)	14.5
Kidney	6	3.0±0.05 (1.5)	1.1±0.05 (7.3)	7.0

^a TFe and HeFe are expressed as mean±SD (CV)

beef with the greatest concentration of ^{55}Fe were: round, ribs, and chuck, together containing more than 60% of the ^{55}Fe found in the muscles (Table 2). With regard to the viscera, the spleen contained the highest concentration of ^{55}Fe per gram; however, the organ that contained more than 55% of the radioactive dose found in the viscera was the liver due to its greater size and weight (Table 3).

Discussion

Reliable information on the content of iron in foods is important for the quantification of iron consumption. Data on intakes of iron is needed to evaluate the nutritional status of populations and thus identify groups at high risk of dietary inadequacy or predict risk of deficiency. Currently, food composition tables are usually lacking information for various nutrients, especially for micronutrients. Hence, the Food and Agricultural Organization (FAO) has recognized this matter needs to be considered a high priority both for the generation and compilation of data and especially for the dissemination of information to professionals in diverse fields to whom it could be of value [23].

There were differences upon comparison of the TFe content of beef observed in this study with composition tables from USA study reports. The values were higher in the USA, ranging between 2.5±0.4 and 2.9±0.5 mg total Fe/100 g, than in this study (1.3 mg/100 g). This difference is likely due to the fact that the measurements in the USA are being taken from cuts of meats already cooked in some way (e.g., beef chuck roast, baked; beef loin/sirloin steak, pan fried; ground beef patty, pan fried). These preparations involve culinary processes whereby moisture is lost from the meat, thus producing increased overall nutrient and iron concentration per unit weight [24, 25]. On the other hand, our values for TFe are similar to results from studies which also had used raw beef: brisket (1.4±0.3), ground beef (1.5±0.3), shank cross cut (1.6±0.4), and top round (1.0±0.5) [26].

The TFe content in beef observed in this study was less in comparison with the Latin American food composition tables on an order of 0.5–1.0 mg/100 mg [27, 28]. This is due to the fact that the latter were developed using data provided by various contributing countries, each of which has its own system of production, classification, and grading of beef cattle, therefore leading to significant variability in muscular composition and intramuscular deposits of micronutrients [29–32]. Nonetheless, except for kidney, the TFe content found in the viscera was comparable to values in the Latin American food composition tables [28, 33].

Currently, little information exists with respect to content of HeFe and the ratio of HeFe to nHeFe. This seems surprising given that heme iron is the most bioavailable form of iron with absorptions ranging between 10% and 30%. On the other hand, nHeFe bioavailability ranges between 1% and 20% due to the many intra- and extraluminal factors affecting its intestinal absorption [34–39]. Beef, among fresh meats, has the highest levels of myoglobin per gram (15 mg) as compared to sheep meat (10 mg), pork (5 mg), and that of poultry (≤ 5 mg) [40]. One study reported that 55–65% of iron in beef is of heme origin [41]. This is consistent with the results from the present study where 65% of iron in meat was determined to be HeFe. The range of the aforementioned study was somewhat large, which can be explained by the fact that the measurements were taken from processed samples, contrasting to the samples in this study which were all taken from raw meat.

To our knowledge, there is no available information with respect to heme iron content in viscera. According to the observations from this study, the organ with the highest level of heme iron is the spleen (72.8% of TFe is HeFe). This is because the spleen's principal functions are erythropoiesis during pregnancy and hemolysis. Another organ with high concentrations of HeFe is the lung (53.8% of TFe is HeFe), probably because it is an organ which is highly irrigated by blood. On the contrary, the liver, the organ with the highest TFe content, has the lowest HeFe (13.6% of TFe is HeFe). This is consistent with the role of the liver in iron storage of which the majority of nHeFe is incorporated as ferritin [42]. The amount of HeFe in liver is also probably due to its level of irrigation by blood.

To our knowledge, there are no published studies with regards to the distribution of ^{55}Fe within the different compartments of livestock animals. It is known that 90% of the radioactive label is distributed in the blood and that the timeframe for incorporation of the isotope into the hemoglobin of the red blood cells is within 15 days of its injection into the bloodstream [43]. In this study, we obtained similar results: 85.5% of the radioisotope was concentrated in the blood 2 months after its injection and less than 3% of the radioactive marker was found in the muscles and viscera. This bears relation to the distribution of iron in the animal organism, given that more than 70% of iron forms part of the blood hemoglobin and the remaining 30% is found in the organs where Fe is stored such as the liver, spleen, and kidney and in the myoglobin molecules in the muscles [44]. As expected, the distribution of ^{55}Fe in the beef meat was in direct proportion to the size of each basic cut and number of muscles of which each cut was composed. For this reason, the three cuts with the highest quantity of ^{55}Fe (round, ribs, and chuck) are precisely those with the greatest muscular mass. But upon conducting muscular analysis by weight, those that presented with the highest ^{55}Fe content per gram of tissue were the diaphragmatic muscles, which also have the highest concentration of heme and total iron. On the other hand, the spleen emerged as the organ with the highest concentration of the radioactive marker per gram of weight and also presented with the highest concentrations of heme and total iron. However, when comparing among organs, the liver accounted for 55% of the marker present in the viscera and the spleen only 20%, given that the liver weighs 5 kg and the spleen only 0.7 kg. This confirms again the role of the liver as the storage organ for iron.

In summary, the results of the present study suggest that beef meat cuts have a low variation in TFe and that HeFe comprises more than 60% of TFe. Furthermore, there was a significant variation in the amounts of TFe and HeFe in viscera, which appears to be related to their diverse functions. The contribution of the study lies in the fact that the reported iron values were determined from whole animals used solely for the purposes of the study and, thus, the optimal iron nutrition status of the animals, their age at sacrifice, breed, and type and form of feeding, among other variables, were all known. These are all important to consider given that, in the majority of studies of this nature, nutrients, minerals, and energy

are all determined from cuts of meat obtained either by purchase or donation. In addition, measures typically come from samples prepared using culinary methods typical of their respective source countries. It should also be noted that this study was conducted on muscles grouped according to US standards for beef.

Acknowledgements CV, MO, MSM, and FP conceptualized and designed the study; CV and FP collected and analyzed the data; CV, DLdR, MO, MSM, and FP interpreted the data; CV, DLdR, and FP drafted and revised the manuscript. The author gratefully acknowledges the technical assistance of Maria Angelica Letelier. This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (Fondecyt) No. 1061060. All authors approved the final version of the manuscript. All authors declare no general, financial, or institutional competing interests.

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