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The effect of proteins from animal source foods on heme iron bioavailability in humans

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ABSTRACT

Forty-five women (35–45 year) were randomly assigned to three iron (Fe) absorption sub-studies, which measured the effects of dietary animal proteins on the absorption of heme Fe. Study 1 was focused on heme, red blood cell concentrate (RBCC), hemoglobin (Hb), RBCC + beef meat; study 2 on heme, heme + fish, chicken, and beef; and study 3 on heme and heme + purified animal protein (casein, collagen, albumin). Study 1: the bioavailability of heme Fe from Hb was similar to heme only (~13.0%). RBCC (25.0%) and RBCC + beef (21.3%) were found to be increased 2- and 1.6-fold, respectively, when compared with heme alone ($p < 0.05$). Study 2: the bioavailability from heme alone (10.3%) was reduced ($p < 0.05$) when it was blended with fish (7.1%) and chicken (4.9%), however it was unaffected by beef. Study 3: casein, collagen, and albumin did not affect the bioavailability of Fe. Proteins from animal source foods and their digestion products did not enhance heme Fe absorption.

1. Introduction

There are two kinds of Fe in the human diet: non-heminic Fe (non-heme Fe), present in plant and dairy-based foods, and heminic Fe (heme Fe), present in foods derived from animal tissue (Sharp & Srai, 2007). The mechanisms of non-heme Fe absorption are widely described and are, generally well understood (Andrews, 1999; Fuqua, Vulpe, & Anderson, 2012). However, research on the mechanisms of heme Fe absorption is not fully understood. Some studies suggest that heme Fe released in the enterocyte is internalized by a mechanism of receptor-mediated pinocytosis (Muller-Eberhard & Fraig, 1993). This mechanism has been demonstrated through studies conducted on Heme Carrier Protein 1 Transports (HCP1) (Beard & Han, 2009; Le Blanc, Garrick, & Arredondo, 2012), and its absorption as a saturable process (Pizarro, Olivares, Hertrampf, Mazariegos, & Arredondo, 2003; West & Oates, 2008). Based on studies performed between 1960 and 1980, heme Fe is poorly absorbed when ingested alone (Conrad, Cortell, Williams, & Foy, 1966), but its absorption increases when ingested as Hb (Conrad et al., 1966; Layrisse & Martínez-Torres, 1972). Absorption also increases when heme Fe is ingested in the presence of foods with high levels of meat proteins (Martínez-Torres & Layrisse, 1971; Martínez-Torres, Romano, & Layrisse, 1981). As a result, it has been postulated that proteins from animal source foods and/or their digestion products maintain heme solubility, favoring heme Fe absorption (Conrad et al., 1966; Martínez-Torres & Layrisse, 1971) through: (a) digestion products from meat proteins, which stimulate heme transfer across the enterocyte and/or, (b) meat proteins that enhance the passage of heme Fe through mucin (Hallberg, Bjorn-Rasmussen, Howard, & Rossander, 1979). Data obtained in Caco-2 cell models supports the theory that globin promotes apical uptake of heme (Follett, Suzuki, & Lonnerdal, 2002) and the possible existence of a protein located in the apical region of enterocytes that negatively regulates the absorption of heme and/or polypeptides that may help in the absorption of heme Fe (Uc, Stokes, & Britigan, 2004). However, in Caco-2 cell studies, reduced heme Fe uptake associated with animal proteins in general has been described, whilst purified animal proteins increased heme Fe uptake (Villarroel, Flores, Pizarro, de Romaña, & Arredondo, 2011). Therefore, the

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involvement of proteins from animal source foods and their digestion products in the absorption of heme is not clear. In this article, we evaluate the effect of dietary proteins from animal source foods on the absorption of heme Fe in humans.

2. Subjects and methods

2.1. Subjects

Forty-five apparently healthy women aged 35–45 years were randomly assigned to three Fe absorption sub-studies (15 in each one). The participants were not taking any medication or vitamins or mineral supplements two months prior to or during the study. None of the participants were blood donors, pregnant or lactating, and all of them were using intrauterine devices or other contraceptive methods at the time of the study. Pregnancy was ruled out using a test for human chorionic gonadotropin in urine. Subjects were characterized by age, weight, height, body mass index and Fe status (biomarkers are described in Section 2.6).

2.2. Ethics

Written, informed consent was obtained from all the volunteers before the studies began. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology, University of Chile and the doses of radioactive isotopes used were approved by the Chilean Commission on Nuclear Energy. Radioisotope labeling of heme Fe protocols in rabbits and calves were approved by the Bioethics Committee of the Institute of Nutrition and Food Technology, University of Chile and by the Bioethics Advisory Committee of the National Fund for Science and Technology (FONDECYT) of Chile.

2.3. Heme Fe labeled with radioactive isotopes

Fe isotopes of high specific activity (^{59}Fe and ^{55}Fe) were used as intrinsic markers of heme Fe (NEN Life Science Products, Inc., Boston), which were injected into the marginal ear vein of five male New Zealand rabbits aged 5 mo and ~3 kg of weight (37 MBq ^{59}Fe diluted in 0.1 mL of a solution of 9 g NaCl/L), and into the jugular vein of two male Holstein Friesian calves aged 4 mo and ~130 kg of weight (740 MBq ^{55}Fe diluted in 3 mL of a solution of 9 g NaCl/L). Fifteen days after the injection of the isotopes, the rabbits and calves received an overdose of anesthetic (10% thiopental at 25 mg/kg I.V); followed by exsanguinations via the jugular route (Hubrecht & Kirkwood, 2010). The blood of the rabbits and calves was received in containers with 0.11 M sodium citrate in a ratio of 9:1 (v/v) citrate:blood and transferred immediately to the laboratory for processing. Heme Fe compounds were prepared using rabbit and calf blood.

2.4. Heme Fe compound preparation

The collected blood was centrifuged at 3207×*g* for 10 min at 10 °C in a refrigerated centrifuge (RC3B Sorvall, Thermo Fisher Scientific, Waltham, MA, USA). Plasma and leukocytes were discarded and red blood cells were washed three times with 9 g/L NaCl. From the red blood cells (RBC), the following was obtained: (a) red blood cell concentrates (RBCC). The RBC of rabbits were frozen in glass balls over 36–48 h and were lyophilized in a freeze dryer (Eyela FD1, Tokyo, Japan) for about 24 h depending on volume. Labeled RBCC with specific activity of 756 kBq ^{59}Fe/mg of Fe was obtained. The labeled RBCC were mixed in dry form with untagged bovine RBC obtained from a calf that was not treated with radioisotopes, resulting in a dose of 37 kBq/5 mg elemental Fe. (b) Hemoglobin (Hb). Bovine RBC were hemolyzed by adding one volume of deionized water, then stroma proteins were precipitated by adding a 20% solution of ammonium sulfate. The final mixture was ultracentrifuged at 20,000×*g* for one hour (Sorvall RC2B, Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was then collected and dialyzed (cutoff point 8000 D) against deionized water to eliminate ammonium sulfate traces. The labeled Hb was mixed in dry form with untagged bovine Hb resulting in a dose of 111 kBq ^{55}Fe/5 mg elemental Fe. (c) Heme. Heme was extracted with the technique described by Labbe and Nishida (1957). RBC from bovine and rabbits were treated with strontium 2% chloride in an acetic acid and acetone solution (1:3), and were heated 10 min to boiling point to separate hemin from globin and other proteins. The final solution was then filtered (Whatman paper filter 1) to eliminate protein residues, and heated again for about 1–2 h under an extraction hood to evaporate acetone and part of the water present in the mixture. The heme started to precipitate when the solution was at room temperature. The final product was washed with an acetic acid water solution (1:1), ethanol, and then diethylether, afterward dried at 37 °C overnight. Labeled purified heme with a specific activity of 1,913 kBq ^{59}Fe and 274 kBq ^{55}Fe/mg of Fe was obtained. The labeled heme was mixed in dry form with untagged bovine heme such that the result was a dose of 37 kBq ^{59}Fe or 111 kBq ^{55}Fe/5 mg elemental Fe.

2.5. Study design (Fig. 1)

Three Fe absorption studies were performed. The doses were administered after 8 h of nocturnal fast, and subjects were not allowed to eat again until 4 h after ingestion of the doses. A sample of 15 subjects/group was calculated in order to detect a 5% difference in the absorption of heme Fe. An alpha equal to 0.05, and 80% power, allowed for an estimated 25% loss to follow-up.

2.5.1. Study 1

This study was conducted to determine the absorption of heme Fe from heme only, RBCC, Hb and RBCC plus beef. The subjects received the same dose of 5 mg of Fe as heme, from the different sources, in gelatin capsules (Reutter Co, Santiago, Chile). On day 1, subjects received ^{55}heme; on day 2, ^{59}RBCC; on day 14, ^{55}Hb; and on day 15, ^{59}RBCC plus 150 g of cow beef (0.79 and 1.39 mg of heme and total Fe/100 g, respectively).

2.5.2. Study 2

This study was designed to measure the effect of heme alone, and heme with fish (*Cilus gilberti*), chicken (*Gallus gallus*) and beef (*Bos taurus*) on heme Fe absorption. The subjects received the same dose of 5 mg of Fe as heme in gelatin capsules (Reutter Co, Santiago, Chile), plus the different meats. On day 1, subjects received

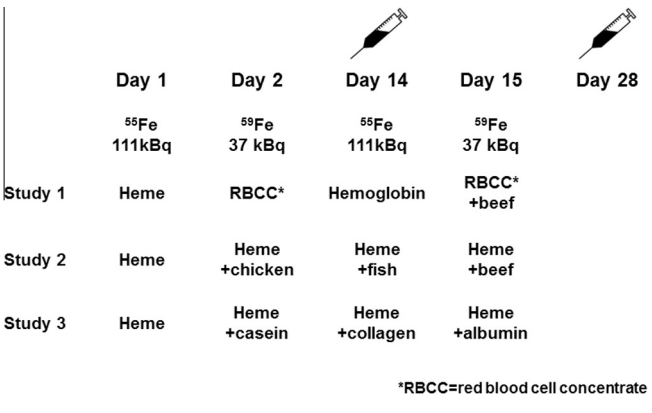

Fig. 1. Experimental design.