Biol Trace Elem Res (2018) 181:38–43
DOI 10.1007/s12011-017-1036-9

Pectin Esterification Degree in the Bioavailability of Non-heme Iron in Women

Angela Jaramillo[1] · **Paulina Molina**[1] · **Lautaro Briones**[2,3] · **Sebastián Flores**[2,3] · **Manuel Olivares**[1] · **Fernando Pizarro**[1]

Received: 27 October 2016 / Accepted: 24 April 2017 / Published online: 12 May 2017

Abstract Pectins are a type of soluble fiber present in natural and processed foods. Evidence regarding the effect of esterification degree of pectins on iron absorption in humans is scarce. In the present study, the effect of pectins with different degrees of esterification on non-heme iron absorption in women was evaluated. A controlled experimental study was conducted with block design, involving 13 apparently healthy, adult women. Each subject received 5 mg Fe ($FeSO_4$) without pectin (control) or accompanied by 5 g citrus pectin, two with a low degree of esterification (27 and 36%), and one with a high degree of esterification (67 to 73%), each on different days. Each day, the 5 mg Fe doses were marked with radioactive ^{59}Fe or ^{55}Fe. Radioactivity incorporated into erythrocytes was determined in blood samples 14 days after the marked Fe doses were consumed. On days 18 and 36 of study, 30 and 20 mL blood samples were obtained, respectively, and blood sample radioactivity incorporated into erythrocytes was determined. Body iron status was determined from blood taken on day 18. Whole body blood volume was estimated for calculate iron bioavailability; it was assumed that 80% of absorbed radioactivity was incorporated into the Hb. All women participants signed an informed consent of participation at baseline. Iron bioavailability (mean geometric ±1 SD) alone (control) was 18.2% (12.3–27.1%), iron + pectin27 was 17.2% (10.2–29.2%), iron + pectin36 was 15.3% (9.5–24.6%), and iron + pectin67 was 19.5% (10.0–38.0%). No statistically significant differences between iron bioavailability (repeated measures ANOVA, $p = 0.22$) were observed. Pectin esterification degree does not influence the bioavailability of non-heme iron in women.

Keywords Iron bioavailability · Pectins · Low esterification degree · High esterification degree · Women

Abbreviations

ANOVA	Analysis of variance
cpm	Counts per minute
ELISA	Enzyme-linked immunosorbent assay
Fe	Iron, chemical element
$FeSO_4$	Ferrous sulfate
$FeCl_3$	Iron chloride
^{55}Fe	Radioactive isotope
^{59}Fe	Radioactive isotope
GI tract	Gastrointestinal tract
Hb	Hemoglobin

✉ Fernando Pizarro
fpizarro@inta.uchile.cl

Angela Jaramillo
anmajaos2013@gmail.com

Paulina Molina
pau.moly@gmail.com

Lautaro Briones
lautarobriones@hotmail.cl

Sebastián Flores
sflores@exedrabio.com

Manuel Olivares
molivare@inta.uchile.cl

[1] Micronutrient Laboratory, Institute of Nutrition and Food Technology, University of Chile, 5524 El Libano, Macul, Santiago, Chile

[2] University of Bio-Bio|, Concepcion, Chile

[3] Exedra Bio Scientific Association, Santiago, Chile

kBq	Kilobecquerel, 10^3 Becquerel (Bq) (unit of radioactivity)
MCV	Mean corpuscular volume
RBC	Red blood cell
Sat%	Transferrin saturation
SF	Serum ferritin
sTfR	Serum transferrin receptor
TIBC	Total iron binding capacity
ZPP	Zinc protoporphyrin

Introduction

Micronutrient deficiency, also known as hidden hunger, affects more than 2 billion people worldwide and, in many cases, coexists with malnutrition and obesity. Among micronutrient deficiencies, the most prevalent nutritional deficiency in developing countries is iron deficiency anemia [1]. A common cause of iron deficiency anemia is the low bioavailability of non-heme iron [2]. Non-heme iron bioavailability is influenced by a number of dietary factors that promote or inhibit its absorption [3]. Dietary fiber has been identified as inhibitory to the absorption of non-heme iron [4], and in some populations, consumption could become a risk factor for inadequate nutritional iron status. It remains unclear whether the inhibitory effect is universal for all types of fiber, or if only certain types demonstrate this result [5].

Pectin is one of many compounds classified as a soluble dietary fiber. Pectins are complex polysaccharides that are part of plant cell walls and are found naturally in fruits, vegetables, legumes, cereals, roots, and nuts [6, 7]. In the food industry, pectins of low and high degrees of esterification are widely added into processed foods [8]. At the medical and pharmacological level, pectins have beneficial effects on gastrointestinal health [9–11], cholesterol-lowering properties [12], and therapeutic effects on colon cancer [13], and are therefore frequently used for these reasons [8].

Despite the health benefits attributed to pectins, it has been described that they may impair the absorption of certain minerals, including iron [14]. In vitro studies show that this type of fiber has the capacity to bind to iron [15, 16] and reduce its availability [17]. In animal models, pectins have been shown to decrease non-heme iron absorption, an effect dependent on their esterification degree [18]. In apparently healthy women, it has been observed that pectins do not modify the bioavailability of iron in a significant manner [19, 20]; however, the effect of esterification degree on iron bioavailability has not been studied in this model. The objective of this study is to determine the effect of citrus pectin esterification degree on non-heme iron bioavailability in women.

Subjects and Methods

Subjects

Fourteen adult women, all apparently healthy, multiparous, contraceptive users, aged between 32 and 46 years, participated. At baseline, it was confirmed that none of the volunteers were pregnant by chorionic gonadotropin urine test (negative). Non-lactating women who were planning a pregnancy in the next 2 years or who had used an iron supplement in the previous 3 months were excluded.

Study Design

This study was designed to determine the effect of esterification degree of a dose of 5 g of citrus pectin on the bioavailability of 5 mg of Fe (as $FeSO_4$). It was reported that 5 mg of iron is the amount contained in a meal [3]. We selected 5 g of pectin given that it has been estimated that an average Western diet provides 4 to 5 g of pectins per day [21].

Subjects were their own controls. Each subject received 5 mg Fe without pectin (control) and the same dose of Fe accompanied by 5 g citrus pectin, two with a low degree of esterification and one with a high degree of esterification, each on different days. The subjects had to attend selected days having fasted for at least 8 h. Instructions were given to all participants to avoid eating or drinking up to 3 h after consuming the compounds. A snack and standard lunch were given.

The 5 mg Fe dose was marked with 37 kBq of ^{59}Fe or 111 kBq of ^{55}Fe (PerkinElmer, Inc., Boston, MA, USA). Prior to ingestion, subjects fasted for 8 h. Each subject drank 50 mL of a solution prepared with 5 mg of Fe ($FeSO_4$), labeled with the appropriate isotope. Immediately after drinking the iron solution, each subject ingested citrus pectin in gelatine capsules. Table 1 shows the compounds used by each day of the study design. All pectins were manufactured by CP Kelco, (Denmark). On day 1 of the study, Genupectin type LM104 AS with 27% esterification and 20% amidation (pectin27) was administered. On day 4, Genupectin type LM101 AS with 36% esterification and 14% amidation (pectin36) was administered. On day 18, Fe was ingested without pectin (control). On day 21, subjects received Genupectin type LM105 with 67 to 73% esterification (pectin67). In order to prevent the pectin dosage delivered on 1 day from interfering with the effect on the bioavailability of Fe of the next dose, 72 h were allotted between intakes, with sufficient time for the pectin to be digested and metabolized [22]. This pectin was chosen because it is used as an ingredient in the food industry.

On days 18 and 36 of study, 30 and 20 mL blood samples were obtained, respectively. Radioactivity incorporated into erythrocytes was determined in blood samples obtained at days 18 and 36. Body iron status was determined from blood taken

Table 1 Compound used by each day of the study design

Day 1 (^{55}Fe) 111 kBq	Day 4 (^{59}Fe) 37 kBq	Day 18 (^{59}Fe) 111 kBq	Day 21 (^{55}Fe) 37 kBq	Day 36
5 mg Fe ($FeSO_4$) + 5 pectin27	5 mg Fe ($FeSO_4$) + 5 g pectin36	5 mg Fe ($FeSO_4$)	5 mg Fe ($FeSO_4$) + 5 g pectin67	
		Blood samples		Blood samples

On days 18 and 36, blood samples with volumes of 30 and 20 mL, respectively, were taken from each subject
pectin27 citrus pectin with 27% esterification, 20% amidation, *pectin36* citrus pectin with 36% esterification, 14% amidation, *pectin67* citrus pectin with 67 to 73% esterification

on day 18. The incorporation process of Fe isotopes into circulating erythrocytes takes 8 days and remains constant until after day 14 [23]. This corresponds to the elapsed time between the intake of compounds and the sample collection. Duplicates of blood samples and six identical samples from ingested solutions were processed according to the technique of Eakins and Brown [24]. Subsequently, the radioactivity was measured in a liquid scintillation counter (Packard 1600TR system TriCarb Scintillation Counter, Meriden CT). Samples were counted a sufficient number of times to ensure an error count smaller than 3%. Using each subject's weight and height taken at baseline, whole body blood volume was estimated (volemia) [25]. To calculate iron bioavailability, the counts per minute per milliliter of ^{55}Fe or ^{59}Fe of sample processed blood was multiplied by the volemia (total cpm circulating). Likewise, this result was divided by total cpm of ^{55}Fe or ^{59}Fe ingested (it was calculated in counts per minute per gram of labeled solution multiplied by grams of solution ingested). The total cpm circulating was divided by total cpm ingested and multiplied by 100. It was assumed that 80% of absorbed radioactivity was incorporated into the Hb, independent of body iron status [23]. To calculate iron bioavailability, the following formula was used (Eq. 1)

$$\text{Fe bioavailability}\ (\%) = \frac{(\text{cpm/mL blood}) \times \text{total blood volume}\ (\text{mL})}{(\text{cpm/mL blood}) \times \text{intake weight}\ (g)} \times \frac{100}{0.8}$$

Blood Samples

Hemoglobin (Hb) and mean corpuscular volume (MCV) were determined by electronic cell count (CELL-DYN 3200, Abbott Diagnostics, Abbott Park, IL, USA). Transferrin saturation (Sat%) was calculated from serum Fe and total iron binding capacity (TIBC) [26]. Zinc protoporphyrin (ZPP) was determined in a hematofluorometer (ZP-M206D, AVIV Biomedical Inc., Lakewood, NJ, USA). Serum ferritin (SF) was determined by enzyme immunoassay (ELISA) [27], as well as serum transferrin receptor (sTfR) (ELISA—Ramco Laboratories Inc., Houston, TX, USA). Body iron content was calculated using the following formula (Eq. 2)

$$\text{Body Iron}\ (\text{mg/kg}) = \left(-\frac{\log\left(\frac{\text{sTfR}\left(\frac{\text{ug}}{\text{L}}\right)}{\text{SF}\left(\frac{\text{ug}}{\text{L}}\right)}\right) - 2.8229}{0.1207} \right)$$

Positive values of body iron (mg/kg) were considered as repletion of iron stores and negative values as iron deficient tissues [19]. Subjects with Hb <120 g/L [28, 29] and two altered iron state parameters were classified as having iron deficiency anemia. Subjects with Hb >120 g/L and two or more altered parameters were classified as having iron deficiency without anemia. The volunteers with serum ferritin <12 μg/L were classified as having iron depleted stores. MCV <80 fL [27], Zpp >70 g/dL RBC [28], Sat <15% [28], and SF <12 μg/L [28] were considered altered parameters. Women with different nutritional iron status included in the analysis because they are representative of the iron nutrition observed in populations of women of fertile age.

Statistics

A sample size of nine subjects was estimated with PRIMER software version 3.02, using the "Power and sample size ANOVA" option. This calculation took into account an alpha of 0.05, a power of 80%, a residual standard deviation of three, a number of treatment groups of four, and a minimum detectable difference of 5%. The number of volunteers was increased to 14, taking into consideration a potential 40% loss of participants that might occur throughout the study due to intake rejection or the presence of diarrhea and/or vomiting, resulting in significant losses of administered compounds.

Statistical analyses were performed in GraphPad Prism software v6.01 (GraphPad Software, Inc., La Jolla, CA, USA). Variables not normally distributed were converted to natural logarithms for statistical tests and reconverted to their original units

Table 2 Subject characteristics

Characteristic	Value
Age (years)[a]	38 ± 4.3
BMI (kg/m^2)[a]	27.0 ± 1.9
Hb (g/L)[a]	151 ± 5
MCV (fL)[a]	87 ± 3
Zpp (μg/dL RBC)[a]	72 ± 13
Transferrin saturation (%)[a]	28 ± 12
Serum ferritin (μg/L)[a]	32 ± 14
sTfR (mg/L)[b]	6.2 (5.6–7.3)
Body Iron (mg/kg)[a]	3.9 ± 2.1

BMI body mass index, *MCV* mean corpuscular volume, *Zpp* zinc protoporphyrin, *Sat* transferrin saturation, *FS* serum ferritin, *sTfR* soluble transferrin receptor

[a] Values are means ± standard deviations

[b] Values are median and interquartile range

to be reported as geometric means with ranges (−1 standard deviation (SD), +1 SD). ANOVA for repeated measures was used to analyze the effect of treatments on iron bioavailability, and the post-hoc Dunnett's test was applied. The bioavailability rate (iron bioavailability by treatment / bioavailability of iron control) for each treatment was calculated and compared. A value of $p < 0.05$ was considered as statistically significant.

Results

Subject Characterization

Thirteen women completed the study, while one volunteer decided not to continue. The participants were aged between 32 and 46 years. Table 2 shows the subjects' characteristics. None of the volunteers presented iron deficiency anemia; however, two had iron deficiency without anemia and one iron depleted stores.

Effect of Pectin Esterification Degree on Non-heme Iron Bioavailability

Table 3 shows the individual values of iron bioavailability. The geometric mean (range ± 1 SD) of the iron bioavailability control ($FeSO_4$) was 18.2% (12.3–27.1%); the bioavailability of pectins with low esterification degree (pectin27 and pectin36) decreased iron bioavailability by 5 and 16%, respectively. In contrast, treatment with pectins of high esterification

Table 3 Effect of pectin esterification degree on non-heme iron bioavailability in women

Subject	Non-heme Fe bioavailability (%)				Bioavailability ratio		
	Day 1 $^{55}FeSO_4$ + pectin27	Day 4 $^{59}FeSO_4$ +pectin36	Day 18 $^{59}FeSO_4$ (control)	Day 21 $^{55}FeSO_4$ + pectin67	Pectin27/ control	Pectin36/ control	Pectin67/ control
1	7.3	14.2	11.9	22.1	0.61	1.20	1.85
2	18.3	11.9	19.8	12.8	0.92	0.60	0.65
3	15.6	10.5	16.6	12.6	0.94	0.63	0.76
4	29.5	27.8	21.5	21.0	1.37	1.29	0.98
5	18.6	13.8	15.3	9.9	1.22	0.90	0.65
6	20.5	14.6	9.0	5.9	2.28	1.62	0.66
7	25.1	14.9	31.4	24.2	0.80	0.48	0.77
8	10.9	14.8	28.2	24.4	0.39	0.53	0.87
9	45.0	41.7	26.2	49.2	1.72	1.59	1.88
10	9.6	7.0	13.4	17.3	0.72	0.52	1.29
11	13.5	18.1	19.4	26.4	0.70	0.93	1.36
12	10.5	9.0	11.9	13.1	0.88	0.76	1.10
13	30.4	23.7	29.8	76.8	1.02	0.80	2.58
Geometric means[a]	17.2	15.3	18.2	19.5	0.95	0.84	1.07
−1 SD	10.2	9.5	12.3	10.0	0.60	0.55	0.68
+1 SD	29.2	24.6	27.1	38.0	1.50	1.28	1.69

ANOVA for repeated measures p = N.S.

pectin27 citrus pectin with 27% esterification, 20% amidation, *pectin36* citrus pectin with 36% esterification, 14% amidation, *pectin67* citrus pectin with 67 to 73% esterification

[a] Values are geometric means ± standard deviations (SD)

degree (pectin67) increased iron bioavailability by 7%; however, these differences were not statistically significant (repeated measures ANOVA $F = 1.59$ $p = 0.22$) (Table 3).

Discussion

This study investigated the effect of 5 g of citrus pectins with low and high esterification degree on the bioavailability of 5 mg iron ($FeSO_4$) in apparently healthy, adult, premenopausal women, in a fasted state. No significant effect on the bioavailability of 5 mg non-heme Fe with citrus pectins of low or high esterification degree was found. Five grams of pectin was used since this amount has been estimated to be about the amount provided by western diets in 1 day [21]. Five milligrams of non-heme Fe was used as it has been calculated as the amount provided by the average lunch or dinner in developing countries [3]. In a study on patients with idiopathic hemochromatosis, Monnier et al. [16] observed an inhibitory effect on the absorption of 1 mg Fe when offered with a dose of 9 g/m^2 pectin. These results cannot be extrapolated to healthy subjects as idiopathic hemochromatosis is a disease characterized by having high intestinal absorption of iron. Furthermore, although Monnier offered compounds in the fasting state, pectin was prepared with ice, which itself can affect the digestive processes of the (gastrointestinal tract) GI tract. Subsequently, Cook et al. [30] did not observe a significant inhibitory effect on the bioavailability of 2.7 mg Fe (as $FeCl_3$), when ingested together with 5 g citrus pectin in the form of wheat flour muffins in apparently healthy women. Jaramillo et al. [20] was not able to find a significant inhibitory effect on the bioavailability Fe using 250 mg citrus pectin (55–70% esterification) and 5 mg Fe ($FeSO_4$). Our results show that doses of 5 g citrus pectin have no significant effect on the bioavailability of 5 mg non-heme Fe in women, apparently healthy, in the fasted state.

Commercially available pectins are classified according to the percentage of methylated carboxyl groups (methyl-esterified or esterified): low methoxyl pectins (<50%) and high methoxyl pectins (>50%) [8]. In an animal model, it has been suggested that the effect of pectin on the bioavailability of Fe depends on its esterification degree. In healthy rats consuming a diet including citrus pectin with a high esterification degree and low molecular weight, non-heme Fe absorption was greater than that of rats fed with a diet containing citrus pectin with a low degree of esterification and high molecular weight [18]. Studies made by Monnier et al. [16] and Cook et al. [30] do not specify the methylation degree of the pectins used. Jaramillo et al. [20] did not observe a significant decrease in the bioavailability of non-heme Fe using high methoxyl citrus pectin (55–70%). This study, like the aforementioned works, did not determine the molecular weight of the pectins used. Amidation has been described as an important characteristic for low methoxyl pectins to bind to calcium and gelify, protecting them from coagulation [31]; however, no interaction with iron has been described. In the present study, two low methoxyl pectins with different degrees of amidation were used and no significant differences in the bioavailability of iron were observed between them. Under these experimental conditions, it was observed that the degree of amidation in low-methoxy pectin has no effect on non-heme iron bioavailability; however, more studies are necessary to fully elucidate the interactions between these molecules.

Acknowledgements We thank Sotiris E. Chaniotakis for editing this manuscript.

Compliance with Ethical Standards All participants signed an informed consent of participation at baseline. Both the protocol and informed consent were previously approved by the Ethics Committee of the Nutrition and Food Technology Institute (INTA) of the University of Chile (Approval Act No. 17, Wednesday, June 27, 2012).

Funding This study was funded by the National Fund for the Scientific and Technological (FONDECYT no. 1130090) and by the National Scientific and Technological Research Commission CONICYT (CONICYT-PCHA/Doctorado Nacional/2014-63140222).

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Zimmermann MB, Hurrell RF (2007) Nutritional iron deficiency. Lancet 370(9586):511–520
2. Lim KH et al (2013) Iron and zinc nutrition in the economically-developed world: a review. Nutrients 5(8):3184–3211
3. Hallberg L (1981) Bioavailability of dietary iron in man. Annu Rev Nutr 1:123–147
4. Debon SJJ, Tester RF (2001) In vitro binding of calcium, iron and zinc by non-starch polysaccharides. Food Chem 73(4):401–410
5. Rossander L (1987) Effect of dietary fiber on iron absorption in man. Scand J Gastroenterol Suppl 129:68–72
6. Baker RA (1997) Reassessment of some fruit and vegetable pectin levels. J Food Sci 62(2):225–229
7. Marlett JA, Vollendorf NW (1993) Dietary fiber content and composition of vegetables determined by two methods of analysis. J Agri Food Chem 41(10):1608–1612
8. Thakur BR, Singh RK, Handa AK (1997) Chemistry and uses of pectin—a review. Crit Rev Food Sci Nutr 37(1):47–73
9. Jenkins DJ et al (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Br Med J 1(6124):1392–1394
10. Sanaka M et al (2007) Effects of agar and pectin on gastric emptying and post-prandial glycaemic profiles in healthy human volunteers. Clin Exp Pharmacol Physiol 34(11):1151–1155
11. Schwartz SE et al (1988) Sustained pectin ingestion: effect on gastric emptying and glucose tolerance in non-insulin-dependent diabetic patients. Am J Clin Nutr 48(6):1413–1417
12. Hillman LC et al (1985) The effects of the fiber components pectin, cellulose and lignin on serum cholesterol levels. Am J Clin Nutr 42(2):207–213

13. Wong TW, Colombo G, Sonvico F (2011) Pectin matrix as oral drug delivery vehicle for colon cancer treatment. AAPS PharmSciTech 12(1):201–214
14. Baig MM, Burgin CW, Cerda JJ (1983) Effect of dietary pectin on iron absorption and turnover in the rat. J Nutr 113(12):2385–2389
15. Fernandez R, Phillips SF (1982) Components of fiber bind iron in vitro. Am J Clin Nutr 35(1):100–106
16. Monnier L et al (1980) Evidence and mechanism for pectin-reduced intestinal inorganic iron absorption in idiopathic hemochromatosis. Am J Clin Nutr 33(6):1225–1232
17. Bosscher D et al (2003) Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. Nutrition 19(7–8):641–645
18. Kim M et al (1996) Pectin with low molecular weight and high degree of esterification increases absorption of 58Fe in growing rats. J Nutr 126(7):1883–1890
19. Cook JD, Flowers CH, Skikne BS (2003) The quantitative assessment of body iron. Blood 101(9):3359–3364
20. Jaramillo A et al (2015) Effect of phytic acid, tannic acid and pectin on fasting iron bioavailability both in the presence and absence of calcium. J Trace Elem Med Biol 30:112–117
21. Pilnik W (1990) Pectin a many splendoured. In: Gums and stabilizers for the food industry. Oxford, Oxford University Press
22. Holloway WD, Tasman-Jones C, Maher K (1983) Pectin digestion in humans. Am J Clin Nutr 37(2):253–255
23. Hosain F, Marsaglia G, Finch CA (1967) Blood ferrokinetics in normal man. J Clin Invest 46(1):1–9
24. Eakins JD, Brown DA (1966) An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isot 17(7):391–397
25. Nadler SB, Hidalgo JH, Bloch T (1962) Prediction of blood volume in normal human adults. Surgery 51(2):224–232
26. Fischer DS, Price DC (1964) A simple serum iron method using the new sensitive chromogen tripyridyl-S-triazine. Clin Chem 10:21–31
27. International Anemia Consultative Group (1985) Measurement of iron status: a report of the International Anemia Consultative Group. The Nutrition Foundation, Washington, DC
28. Centers for Disease Control and Prevention CDC (1998) Recommendations to prevent and control iron deficiency in the United States. MMWR 47(RR-3):1–29
29. World Health Organization (2001) Iron deficiency anaemia: assessment, prevention, and control. A Guide for Programme Managers. WHO, Geneva
30. Cook JD et al (1983) Effect of fiber on nonheme iron absorption. Gastroenterology 85(6):1354–1358
31. Reitsma JCE, Thibault JF, Pilnik W (1986) Properties of amidated pectins. I. Preparation and characterization of amidated pectins and amidated pectic acids. Food Hydrocoll 1(2):121–127