

Iron bioavailability in daily meals of pregnant women

INES BANJARI – DANIELA KENJERIĆ – MILENA L. MANDIĆ

SUMMARY

Pregnancy presents a critical period for both woman and child, and proper iron status is especially important to prevent adverse pregnancy outcomes. For iron the problem almost exclusively lies in nutritional deficiency, since meat presents the best food source of iron, and energy and iron intake are in direct correlation. That is even more emphasized in times of increased needs for iron, like in pregnancy. The aim was to determine whether and in what scale does meals composition influence iron bioavailability, with an insight in changes throughout pregnancy. Randomized prospective observational study encompassed 222 singleton pregnancies from Eastern Croatia. Nutrition quality analysis by multiple 24-hour dietary recall, during 2010–2011, showed low intakes of energy and proteins ($p < 0.001$) through trimesters. Plant foods were the main source of iron contributing more than 80% to total iron. Other inhibiting factors (tannic acid $p = 0.024$, calcium $p < 0.001$) showed significant influence on iron bioavailability. Still, physiologic adaptation during pregnancy resulted in slight but significant increase in absorbed iron (from 1.20 mg in the 1st to 1.33 mg in the 3rd trimester, $p = 0.035$). The results imply the need for the educational intervention programmes, aiming at food choices during pregnancy.

Keywords

pregnancy; nutrition quality; iron bioavailability; absorption; inhibition

Iron bioavailability over and over again draws vast attention. It is well known that various food sources have different contents of bioavailable iron or, to be more specific, contain different amounts of iron that can be readily absorbed in duodenum [1]. Still, other conditions in the gut should be also considered, since they additionally influence the amount of iron that will be absorbed. If we presume absence of any serious pathology affecting the gut (e.g. gastritis, worm infestation, coeliac disease, gut neoplasia, irritable bowel disease, ulcerative colitis), then the iron deficiency (ID) and iron deficiency anemia (IDA) have to be considered. This one of the most common nutritional deficiencies worldwide affects 35% to 75% of pregnant women in developing countries (on average 56%), and 18% in developed countries [2]. IDA reflects different stages of depleted iron stores in the body (liver and bone marrow), which consequently causes low hemoglobin synthesis

leading towards symptoms of low oxygen delivery [1–3].

Iron in foods is present as heme and non-heme, differing by solubility, sources and absorption level [1]. Foods that are part of the usual, everyday meals have low iron contents and low bioavailability. So only 10% to 20% of total iron intake is absorbed, but the absorption percentage is higher at IDA [2–5]. The reason for low absorption lies in numerous inhibitors of iron absorption, like phytic and oxalic acid, starch, polyphenols (i.e. tannins from coffee and tea), egg white, calcium, other minerals (e.g. zinc), and numerous medicaments that diminish gastric secretion (e.g. antacids) [3, 6–8].

Since intake of iron is in direct correlation with energy intake (on every 4184 kJ comes about 6 mg of iron) [1, 9–11], risk of ID is especially high in times when iron needs exceed the energy needs [3]. One of the reasons lies in the disproportion

Ines Banjari, Daniela Kenjerić, Milena L. Mandić, Department of Food and Nutrition Research, Faculty of Food Technology Osijek, University of Osijek, F. Kuhača 18, Osijek, HR-31000, Croatia.

Corresponding author

Ines Banjari, tel.: +385 31 224 300, fax: +385 31 207 115, e-mail: ines.banjari@ptfos.hr

between slight increase in energy needs during gestation and the need for iron of 27 mg per day [12]. Also, physiology of pregnancy requires additional 800 mg of circulating iron during gestation [1, 9–11]. Hence, high incidence of anemia during pregnancy and lactation is foreseen [13, 14]. It is of utmost importance to monitor iron status and iron intake during pregnancy since IDA presents a risk factor for pregnancy outcomes, such as preterm delivery, small-for-gestational-age births and low birth weight infants [3, 13–15]. Therefore, the aim of this study was to determine the quality of daily diet of a representative group of pregnant women through pregnancy, with an emphasis on iron bioavailability.

MATERIALS AND METHODS

To ensure that set aims could be covered, an observational randomized longitudinal study was chosen. For the randomized sample, the inclusion criterion was healthy pregnancy within 12 weeks of gestation (i.e. 1st trimester), followed in two general gynecologist offices in the area of the city of Osijek, Eastern Croatia. Early pregnancy was selected since statistical data for past several years show that a portion of around 88% of women pregnancies is confirmed within 12 weeks of gestation [16, 17]. The number of 251 pregnant women were enrolled during one year period and monitored (throughout gestation to labour) from 2010 till the end of 2011. Total of 29 women were excluded for forced abortion, preterm labour, newborn death, twin pregnancy, or lack of data. The dropout was 11.6%. Overall number of pregnant women was 222. The study was approved by the Ethical committee of Faculty of Food Technology Osijek; an informed consent was obtained for all participating pregnant women. Recruited population was by all demographic and socio-economic characteristics representative for the overall population of pregnant women from the encompassed area [16, 17], and presented 14.8% of the total population of pregnant women for the study period.

Nutrition quality assessment was done by 24-hour dietary recall in a multi-pass protocol, and was repeated once during each trimester. Information that could influence under- or over-reporting was taken into account (e. g. avoiding weekends, holidays and special occasions, never completed on the same days, all seasons were covered). Computer program NutriPro (Faculty of Food Technology Osijek, Osijek, Croatia), which uses National Composition tables [18], was used to cal-

culate the energy intake and the intake of macronutrients and micronutrients, and the results were compared to the recommended intake in pregnant women [12]. Iron bioavailability assessment was done according to the protocol by HALLBERG and HULTÉN [19]. The algorithm considers the promoting influence of vitamin C, meat and fish, as well as inhibiting influence of phytates, polyphenols (i.e. tannic acid), calcium, soya proteins and eggs. The protocol was as follows [19]:

- 1 cup of coffee (150 ml) equals 15 mg of tannic acid,
- 1 cup of tea (200 ml) equals 30 mg of tannic acid,
- 1 g of meat (includes all types of meat, poultry and fish) in a meal equals 1.3 g of raw meat,
- 3.35 mg of phytates equals 1 mg of phytate-phosphorus (phytate-P) [19].

Intakes of phytates, calcium and vitamin C were taken from the calculations done by NutriPro, and after considering all above mentioned factors the percentage of absorption was gained. This percentage was applied on total iron intake from foods (calculated by NutriPro) and the final result represented the amount of absorbed iron in milligrams.

Statistical analysis was done with the software tool Statistica 8.0 (StatSoft, Tulsa, Oklahoma, USA), at significance level $p = 0.05$. Normality of data distribution was tested by the non-parametric Kolmogorov-Smirnov test for the comparison of medians and arithmetic mean, and histograms plotting. Friedman test was used to test three dependent variable groups, since overall data did not show normal distribution. MS Office Excel (Microsoft, Redmond, Washington, USA) was used for other calculations and graphs.

RESULTS AND DISCUSSION

Overall diet quality

Analysis of 24-hour recalls showed statistically significantly higher intakes of all macronutrients and energy as pregnancy progressed ($p < 0.001$; Tab. 1). Despite the statistically significant rise in intakes of all observed components from the 1st up to the 3rd trimester, 2/3 of pregnant women did not satisfy the recommended intake neither for energy or proteins (Fig. 1). Considering relation between iron and energy intake [4, 6, 7, 19], and between meat and meat products, iron and proteins [19], a low intake of iron in a minimum of 2/3 pregnant women can be expected.

Tab. 1 also shows that the intake of fibres sta-

Tab. 1. Average daily intake of energy and macronutrients during pregnancy.

Energy and macronutrients	Trimester			<i>p</i>
	1st	2nd	3rd	
Energy [kJ]	7780 (6274–9354)	8411 (6986–10274)	9826 (8026–11996)	< 0.001
Proteins [g]	58.7 (44.6–74.5)	59.3 (50.5–75.9)	71.7 (57.9–90.5)	< 0.001
Lipids [g]	73.2 (53.0–96.9)	80.6 (61.2–106.2)	101.5 (70.4–135.1)	< 0.001
Carbohydrates [g]	237.0 (196.1–299.8)	257.5 (206.0–310.5)	290.6 (229.2–347.1)	< 0.001
Fibre [g]	20.5 (15.0–26.3)	20.6 (15.5–26.3)	22.9 (16.7–28.5)	< 0.001

Friedman's test ($n = 222$). Values of median daily intake are given. Values in parentheses represent a range of 25–75% of daily intake.

tistically significantly rose through trimesters (from 20.5g in the 1st to 22.9g in the 3rd trimester, $p < 0.001$). Despite that, the intake of fibres was below the recommended intake of 28 g per day [12] for 81.5% of pregnant women in the 1st trimester, falling in the last trimester to 70.7% of pregnant women with insufficient intake of fibres (Fig. 1). Higher intake of fibres is known to be related to better nutrition quality in terms of consumption of wholegrain cereals [20–24], and fruits and vegetables [24]. By observing other macronutrients, change in the distribution of separate macronutrients to the total energy intake could be noted. Share of proteins in the total energy intake was within the recommended range of 10–15% [12] through all trimesters, starting from 12.6%, then 11.8% and 12.2% in the 3rd trimester. On the other hand, share of fats rose (35.9%, 37.0%

and 38.6% through trimesters, respectively), while the share of carbohydrates in the total energy decreased (51.0%, 51.2% and 49.2% through trimesters, respectively).

Earlier commented results showed that despite the general belief of positive shifts in the nutrition quality during pregnancy [25], pregnant women did not change their nutrition quality significantly through pregnancy. Therefore, efforts should aim at the education programmes for pregnant women, since they have numerously shown long-term success [15, 26–28].

Daily intake of iron from foods

Mean daily intake of iron from foods rose statistically significantly through gestation (from 9.5 mg in the 1st, 10.1 mg in the 2nd to 11.2 mg in the 3rd trimester, $p < 0.001$). These results are in

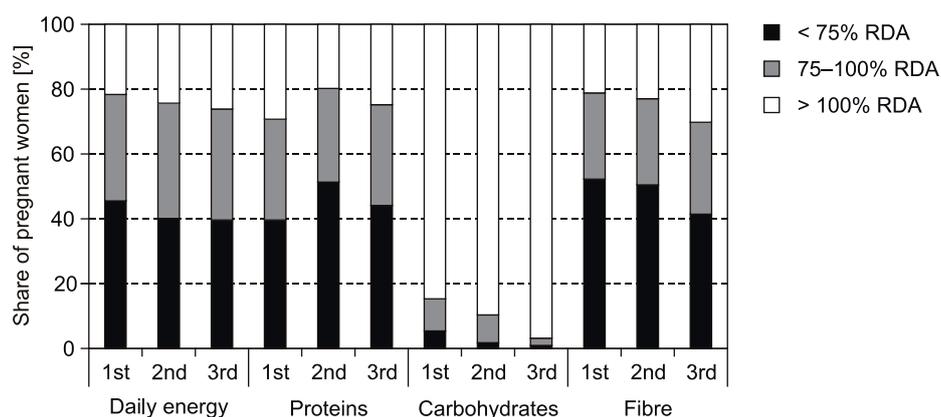


Fig. 1. Intake of energy and macronutrients expressed as percentage of satisfied recommended intake during trimesters.

Intakes are expressed as percentage of satisfied recommended intake (RDA) and give information regarding the share of pregnant women ($n = 222$) satisfying up to 75% of RDA, 75–100% RDA and more than 100% RDA. Recommendations used are as follows [12]: for energy in the 1st trimester 10 054 kJ per day, in the 2nd 10 347 kJ per day, and in the 3rd 11 945 kJ per day; for proteins in the 1st trimester 71 g per day, in the 2nd 81 g per day and in the 3rd 91 g per day; for carbohydrates 175 g per day during all trimesters; for fibres 28 g per day during all trimesters.

Tab. 2. Average daily intake of iron of different food sources, and assessment of iron bioavailability during pregnancy.

Dietary factors affecting iron bioavailability	Trimester			
	1st	2nd	3rd	<i>p</i>
Fe total [mg]	9.5 (7.5–12.4)	10.1 (7.8–13.3)	11.2 (8.7–14.6)	< 0.001
Fe from animal foods [mg]	1.50 (0.63–2.70)	1.66 (0.68–2.73)	1.86 (0.96–3.30)	0.042
Fe from plant foods [mg]	7.61 (6.01–10.33)	7.98 (5.99–10.78)	8.92 (6.83–11.61)	< 0.001
Fe absorbed [mg]	1.20 (0.87–1.71)	1.29 (0.91–1.81)	1.33 (0.99–1.82)	0.035
Meat intake [g]	94.4 (56.8–161.3)	85.4 (55.0–136.5)	91.0 (57.2–149.4)	ns
Calcium [mg]	698 (498–952)	684 (523–938)	761 (610–1051)	< 0.001
Vitamin C [mg]	131 (64–217)	135 (70–229)	127 (63–223)	ns
Tannic acid equivalents from coffee and tea [mg]	11.0 (0.0–22.0)	15.0 (0.0–22.0)	11.0 (0.0–20.5)	0.024
Phytates [g]	169.3 (69.6–392.8)	177.3 (85.0–380.4)	194.6 (101.5–429.1)	ns

Friedman's test ($n = 222$). Values of median daily intake are given. Values in parentheses represent a range of 25–75% of daily intake. Values for iron from animal and plant foods are presented as percentage of total intake of iron. ns – not significant

accordance with many others [6, 14, 29, 30]. Regardless of this rising trend, the results emphasize an important information that the daily intake of iron was unsatisfactory, supplying only from 35.2% in the 1st trimester up to 41.5% in the 3rd trimester of the daily need for iron in pregnancy of 27 mg per day [12] (Tab. 2). SHOBEIRI et al. [29] reported the intake of iron in Indian pregnant women of around 60% of the dietary recommended intake.

Iron bioavailability in daily meals

A proper combination of foods is a key to maximum absorption of iron through pregnancy, proven by dietary interventions on long-term improvement in iron status [28]. BARETT et al. [31] showed that the iron absorption rose through pregnancy and presented a normal physiologic process in pregnancy, reaching a maximum by the end of gestation, falling again postpartum to the starting level (of around 11%). Simultaneously, individual iron stores should be considered, i.e. presence of ID or IDA [2–5]. Our results confirm that the amount of absorbed iron follows a rising trend towards the end of pregnancy (Tab. 2). Even if the amount of absorbed iron is quite low, the rising trend is statistically significant ($p = 0.035$), the amount being the highest in the 3rd trimester with 1.33 mg of absorbed iron.

The reason for such a low amount of absorbed iron partially lies in the fact that plant foods present the main source of dietary iron in the studied population of pregnant women. Contribution of plant foods to the overall intake of iron in studied population was higher than 80% throughout gestation (Tab. 2), which is in accordance with

results of other authors [3, 6–8, 28, 32, 33].

The low amount of absorbed iron resulted from the meat intake of around 90 g a day, consumption of tannic acid from coffee and tea ($p = 0.024$), and high intake of phytates (Tab. 2). Heme iron contributed to the overall intake of iron with 15.8%, 16.4% and 16.6%, respectively, through gestation (Tab. 2). These low percentages of heme iron can be explained by the fact that, for the largest part of the studied population, chicken were the main meat source. For the past two decades, the trend of higher consumption of poultry of more than 50% with the simultaneous fall in beef consumption of 40% were noted, therefore the amount of heme iron was significantly lower [33]. Besides that, chicken meat does not contain heme iron [34], but still “meat factor” effect shows positive influence on iron absorption [35].

Consumption of cereals is very important since they present an important part of everyday meals in the studied area [20], they are the main source of the most inhibiting absorption factor, phytates [19, 36] and, according to JOHNSTON et al. [33], present the source of non-heme iron most importantly contributing to its overall intake.

Another important influence showed coffee and tea that were also widely consumed on a daily basis. Their inhibiting effect is known to be related to polyphenols (galic, tannic and chlorogenic acids). Tea shows a higher reduction rate (by 75–80% for cca 200 ml) than coffee (by 60% for cca 150 ml). However, the results show that around 100 g of meat reduced their inhibiting effect by 50% [19, 37, 38]. Since our results showed the intake of meat below 100 g (Tab. 2), its

influence on iron absorption was probably mostly related to earlier mentioned “meat factor”.

Also, intake of calcium was in the highest range (more than 600 mg per day) and statistically significantly rose by the end of gestation ($p < 0.001$; Tab. 2). Intake of calcium in this range was found to have the maximum inhibiting effect on iron absorption [19, 39–41]. On the other hand, intake of vitamin C as the most promoting factor in iron absorption, especially in the presence of the most powerful inhibitors in a meal [19, 42], did not differ significantly through trimesters (Tab. 2). Still, its intake was generally above the recommended 85 mg per day [12] and affected iron absorption positively, as shown by others [19,42].

Strengths and limitations of the study

This study gave representative data for the studied population of pregnant women. Even though these are observational data, they give insight in overall diet quality and iron intake during gestation. These data are really important since no similar research was conducted on pregnant women in any part of Croatia. Also, these results can serve as guidelines for other researchers, both in Croatia and other countries or areas with similar dietary and lifestyle habits.

The 24-hour dietary recall is considered a standard method, but food frequency questionnaire (FFQ) is highlighted as another preferable method for studies on dietary intake of iron. Combining these methods would give better insight on diet quality and iron intake of studied population. However, FFQ is time-consuming and costly, therefore not preferable for population studies. Still, these results would present a good basis for educational intervention study. Intervention studies are also costly and ask for additional time and involvement of more researches. Costs rise in case of long period of intervention, as in this case when preferable duration would be during whole gestation. Cost effectiveness asks for a smaller group or a specific intervention programme. Therefore, smaller test educational intervention as proposed would give insight in adequacy and justifiability for its application on a larger, national scale. If the intervention shows results in positive outcomes, it would present a ground basis for the making of Croatian national guidelines for pregnant women. So, the final outcome would be a prevention programme aiming at dietary and lifestyle habits of pregnant women on a national level.

In conclusion, despite the general belief, our research revealed that pregnancy was not related to better nutritional habits. Intake of energy and

proteins was low throughout gestation. Intake of iron from foods increased during pregnancy, but it was low and did not satisfy recommendations in any stage of gestation. Altogether, more efforts should be directed towards educational programmes for pregnant women. These educational programmes should aim at correcting nutritional habits, teaching women how to combine foods to gain maximum iron and other nutrients crucial for normal pregnancy outcomes.

Acknowledgements

The authors wish to thank all women who decided to participate in this study and shared the experience of expecting new life with us. Also, thanks goes to doctors of obstetrics and gynaecology, and to nurses at the gynecologist offices.

REFERENCES

1. Boulpaep, E. L. – Boron, W. F.: Medical physiology. Saunders : Elsevier, 2006. 1267 pp. ISBN 978-1-4160-3115-4.
2. Iron deficiency anaemia: assessment, prevention, and control – A guide for programme managers. WHO/NHD/01.3, 2001. Geneva: World Health Organization, 2001. 114 pp.
3. Zimmermann, M. B. – Hurrell, R. F.: Nutritional iron deficiency. *Lancet*, 370, 2007, pp. 511–520.
4. Hurrell, R. – Egli, I.: Optimizing the bioavailability of iron compounds for food fortification. In: Nutritional anemia. Basel: Sight and Life Press, 2007, pp. 77–97.
5. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation Bangkok, Thailand. Rome: Food and Nutrition Division FAO, 2001. 286 pp.
6. Milman, N.: Iron in pregnancy – a delicate balance. *Annals of Hematology*, 85, 2006, pp. 559–565.
7. Thompson, B.: Food-based approaches for combating iron deficiency. In: Nutritional anemia. Basel: Sight and Life Press, 2007, pp. 337–358.
8. Tapiero, H. – Gaté, L. – Tew, K. D.: Iron: deficiencies and requirements. *Biomedicine and Pharmacotherapy*, 55, 2001, pp. 324–332.
9. Adamson, J. W.: Iron deficiency and other hypoproliferative anemias. In: Harrison's principles of internal medicine. 17th edition. New York: McGraw Hill Medical, 2008, pp. 628–634.
10. Berger, J. – Wieringa, F. T. – Lacroux, A. – Dijkhuizen, M. A.: Strategies to prevent iron deficiency and improve reproductive health. *Nutrition Reviews*, 69, 2011, pp. S78–S86.
11. Wheeler, S.: Assessment and interpretation of micronutrient status during pregnancy. *Proceedings of the Nutrition Society*, 67, 2008, pp. 437–450. DOI 10.1017/S0029665108008732.

12. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Washington, D. C.: National Academy Press, 2002. 1319 pp.
13. Scholl, T. O.: Maternal iron status: relation to fetal growth, length of gestation, and iron endowment of the neonate. *Nutrition Reviews*, 69, 2011, pp. S23–S29.
14. Lee, J.-I. – Kang, S. A. – Kim, S.-K. – Lim, H.-S.: A cross sectional study of maternal iron status of Korean women during pregnancy. *Nutrition Research*, 22, 2002, pp. 1377–1388.
15. Viteri, F. E.: Iron endowment at birth: maternal iron status and other influences. *Nutrition Reviews*, 69, 2011, pp. S3–S16.
16. Childbirths and abortions in healthcare institutions in Osijek-Baranja County in 2009. Osijek: Institute of public health Osijek-Baranja County, 2010. 17 pp.
17. Childbirths in healthcare institutions in Osijek-Baranja County in 2010. Osijek: Institute of public health Osijek-Baranja County, 2011. 19 pp.
18. Kaić-Rak, A. – Antonić, K.: Tablice o sastavu namirnica i pića. Zagreb: Zavod za zaštitu zdravlja SR Hrvatske, 1990. 46 pp.
19. Hallberg, L. – Hultén, L.: Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition*, 71, 2000, pp. 1147–1160.
20. Banjari, I. – Kenjerić, D. – Mandić, M. L.: Cereals and their products as source of energy and nutrients in early pregnancy. In: Proceedings of the 6th International Congress Flour-Bread'11. Osijek: Faculty of Food Technology Osijek, 2012, pp. 110–117. <<http://www.cabdirect.org/abstracts/20123355616.html>>. ISSN 1848-2562.
21. Cecić, I. – Colić Barić, I. – Kuvačić, S. – Batinić, M.: Diet quality and grains intake in Croatian pregnant women. In: Proceedings of the 5th International Congress Flour-Bread '09. Osijek: Faculty of Food Technology Osijek, 2010, pp. 463–470. <<http://www.cabdirect.org/abstracts/20103326886.html>>. ISBN 978-953-7005-21-4.
22. Buss, C. – Nunes, M. A. – Camey, S. – Manzolli, P. – Soares, R. M. – Drehmer, M. – Giacomello, A. – Duncan, B. B. – Schmidt, M. I.: Dietary fibre intake of pregnant women attending general practices in southern Brazil – The ECCAGE Study. *Public Health Nutrition*, 12, 2008, pp. 1392–1398.
23. Snook Parrott, M. – Bodnar L. M. – Simhan, H. N. – Harger, G. – Markovic, N. – Roberts, J. M.: Maternal cereal consumption and adequacy of micronutrient intake in the periconceptional period. *Public Health Nutrition*, 12, 2008, pp. 1276–1283.
24. Radhika, G. – Sudha, V. – Mohan Sathya, R. – Ganesan, A. – Mohan, V.: Association of fruit and vegetable intake with cardiovascular risk factors in urban sputh Indians. *British Journal of Nutrition*, 99, 2008, pp. 398–405.
25. Rifas-Shiman, S. L. – Rich-Edwards, J. W. – Kleinman, K. P. – Oken, E. – Gillman, M. W.: Dietary Quality during pregnancy varies by maternal characteristics in Project Viva: A US Cohort. *Journal of the American Dietetic Association*, 109, 2009, pp. 1004–1011.
26. Verbeke, W. – De Bourdeaudhuij, I.: Dietary behaviour of pregnant versus non-pregnant women. *Appetite*, 48, 2007, pp. 78–86.
27. Black, M. M. – Quigg, A. M. – Hurley, K. M. – Reese Pepper, M.: Iron deficiency and iron-deficiency anemia in the first two years of life: strategies to prevent loss of developmental potential. *Nutrition Reviews*, 69, 2011, pp. S64–S70.
28. Patterson, A. J. – Brown, W. J. – Roberts, D. C. K. – Seldon, M. R.: Dietary treatment of iron deficiency in women of childbearing age. *American Journal of Clinical Nutrition*, 74, 2001, pp. 650–656.
29. Shobeiri, F. – Begum, K. – Nazari, M.: A prospective study of maternal hemoglobin status of Indian women during pregnancy and pregnancy outcome. *Nutrition Research*, 26, 2006, pp. 209–213.
30. Petrakos, G. – Panagopoulos, P. – Koutras, I. – Kazis, A. – Panagiotakos, D. – Economou, A. – Kanellopoulos, N. – Salamalekis, E. – Zabelas, A.: A comparison of the dietary and total intake of micronutrients in a group of pregnant Greek women with the dietary reference intakes. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 127, 2006, pp. 166–171.
31. Baret, J. F. R. – Whittaker, P. G. – Williams, J. G. – Lind, T.: Absorption of non-haem iron from food during normal pregnancy. *British Medical Journal*, 309, 1994, pp. 79–82.
32. Hoppe, M. – Sjöberg, A. – Hallberg, L. – Hultén, L.: Iron status in Swedish teenage girls: impact of low dietary iron bioavailability. *Nutrition*, 24, 2008, pp. 638–645.
33. Johnston, J. – Prynne, C. J. – Stephen, A. M. – Wadsworth, M. E. J.: Haem and non-haem iron intake through 17 years of adult life of a British Birth Cohort. *British Journal of Nutrition*, 98, 2007, pp. 1021–1028.
34. Hallberg, L. – Hultén, L.: Perspectives on iron absorption. *Blood Cells, Molecules and Disease*, 29, 2002, pp. 562–573.
35. Reddy, M. B. – Hurrell, R. F. – Cook, J. D.: Meat consumption in a varied diet marginally influences nonheme iron absorption in normal individuals. *Journal of Nutrition*, 136, 2006, pp. 576–581.
36. Kristensen, M. B. – Tetens, I. – Alstrup Jørgensen, A. B. – Dal Thomsen, A. – Milman, N. – Hels, O. – Sandström, B. – Hansen, M.: A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread. *European Journal of Nutrition*, 44, 2005, pp. 334–340.
37. Hurrell, R. F. – Reddy, M. – Cook, J. D.: Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition*, 81, 1999, pp. 289–295.
38. Manach, C. – Scalbert, A. – Morand, C. – Rémésy, C. – Jiménez L.: Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 2004, pp. 727–747.
39. Ziegler, E. E.: Consumption of cow's milk as a cause

- of iron deficiency in infants and toddlers. *Nutrition Reviews*, *69*, 2011, pp. S37–S42.
40. Lynch, S. R.: The effect of calcium on iron absorption. *Nutrition Research Reviews*, *13*, 2000, pp. 141–158.
41. Glerup, A. – Rossander-Hulthen, L. – Gramatkovski, E. – Hallberg, L.: Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *American Journal of Clinical Nutrition*, *61*, 1995, pp. 97–104.
42. Cook, J. D. – Reddy, M. B.: Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *American Journal of Clinical Nutrition*, *73*, 2001, pp. 93–98.

Received 19 April 2013; revised 29 May 2013; accepted 14 June 2013; published online 18 September 2013.