

IRON BIOAVAILABILITY IN PRENATAL MULTIVITAMIN SUPPLEMENTS WITH SEPARATED AND COMBINED IRON AND CALCIUM

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Abstract

Objective: To compare iron absorption of a prenatal multivitamin supplement containing both iron and calcium (HICA) to that of another multivitamin containing a lower iron dose and no calcium (LI).

Methods: In a crossover study, serum iron was measured in 12 healthy women administered HICA and LI separately on 2 different occasions. Blood samples were taken at 0, 1, 2, 3, 4, 6, and 8 hours after administration of each supplement.

Results: The values of the area under the concentration-time curve (AUC) were not significantly different between LI ($79.1 \pm 36.0 \mu\text{M}^*\text{h}$) and HICA ($91.4 \pm 50.4 \mu\text{M}^*\text{h}$) ($P = .37$). After standardizing the AUC for dose, the relative absorption over the 8-hour time period was significantly higher for LI ($2.3 \pm 1.0 \mu\text{M}^*\text{h}/\text{mg}$) than for HICA ($1.5 \pm 0.8 \mu\text{M}^*\text{h}/\text{mg}$) ($P = .021$).

Conclusion: The absorption of iron from a low-iron-containing supplement was similar to that from a supplement with almost twice the amount of iron, due possibly to the exclusion of calcium in the LI product. Thus, while offering similar amounts of iron, the LI supplement may be better tolerated by women who are sensitive to iron-induced adverse effects.

Résumé

Objectif : Comparer l'absorption du fer que permet un supplément multivitaminique prénatal contenant du fer et du calcium (HICA) à celle que permet un supplément multivitaminique contenant une dose moindre de fer et ne contenant pas de calcium (LI).

Méthodes : Dans le cadre d'une étude croisée, le taux sérique de fer a été mesuré chez 12 femmes en santé auxquelles l'on avait administré du HICA et du LI séparément, à deux occasions distinctes. Des échantillons de sang ont été prélevés

à 0, 1, 2, 3, 4, 6 et 8 heures après l'administration de chacun des suppléments.

Résultats : Les valeurs de l'aire sous la courbe concentration-temps (ASC) du LI ($79,1 \pm 36,0 \mu\text{M}^*\text{h}$) et du HICA ($91,4 \pm 50,4 \mu\text{M}^*\text{h}$) ($P = 0,37$) ne se sont pas avérées considérablement différentes les unes des autres. Après normalisation de l'ASC en fonction de la dose, l'absorption relative au cours de la période de huit heures a été considérablement plus élevée dans le cas du LI ($2,3 \pm 1,0 \mu\text{M}^*\text{h}/\text{mg}$) que dans celui du HICA ($1,5 \pm 0,8 \mu\text{M}^*\text{h}/\text{mg}$) ($P = 0,021$).

Conclusion : L'absorption du fer issu d'un supplément à faible teneur en fer a été semblable à celle du fer issu d'un supplément contenant presque deux fois plus de fer, ce qui est peut-être attribuable à l'absence de calcium dans le produit LI. Ainsi, bien qu'il permette l'obtention de quantités semblables de fer, il est possible que le supplément LI soit mieux toléré par les femmes qui sont sensibles aux effets indésirables provoqués par le fer.

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INTRODUCTION

The increasing need for iron during pregnancy stems from the increasing amount of iron transferred to both the placenta and the growing fetus, as well as the need to expand the red blood cell mass.¹ Failure to meet the increased demands of iron due to insufficient intake, inadequate or impaired absorption of iron, or chronic blood losses, can lead to an iron-deficient state that can later manifest as anemia.¹ In developed countries, 10% of women between the ages of 15 and 45 are anemic,² and many other women are in an iron-deficient state. Canadian data on the iron intake of women of child-bearing age report average dietary intake below the reference nutrient intake.^{3–6} Small-scale Canadian studies suggest that many pregnant women suffer from iron deficiency.⁷ Several studies from New Brunswick found that anemia was common in pregnant women (21%–24%), including relatively well-educated, middle- and upper-middle-class families.^{8–10} In Nunavik, an arctic region of Quebec, the prevalence of anemia was found to be 40%.¹¹

Key Words

Iron, biological availability, Materna, PregVit, vitamins, dietary supplements, prenatal care

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Because it is generally felt that diet alone cannot meet the increased requirements for iron in pregnancy,¹² the subcommittee on Maternal Nutrition of the National Academy of Sciences has recommended a daily dose of 27 mg of elemental iron in a form of ferrous iron throughout pregnancy.¹³

A common complication of iron supplementation is iron-associated adverse effects when given at high doses. The lowest observed adverse effect level for iron, set at 60 mg per day, was associated with a higher risk of constipation and gastrointestinal effects compared to a lower iron dose.¹⁴ The common side effects associated with high oral iron intake, such as nausea, constipation, fatigue, diarrhea, and headache, may impede daily consumption of these supplements.^{15,16} Alward and Kevany found that intestinal adverse effects of iron may cause up to 40% of pregnant women to compromise on compliance.¹⁷ Another group reported that 10% of pregnant women discontinued the use of iron because of its adverse effects.¹⁵ Reducing the dosage of iron in prenatal supplements may reduce the occurrence and severity of the adverse effects associated with iron, thereby improving daily usage of supplements.¹⁸

The inhibitory effect of calcium on iron absorption, when taken concurrently, has been well documented.¹⁹⁻²¹ Avoiding

this mineral-mineral interaction could potentially lead to a relative increase in iron bioavailability. Due to the inhibitory effect of calcium on iron absorption, Health Canada encourages women to take these mineral supplements at different times.²² The objective of the present study was to compare iron absorption from a prenatal supplement with iron and calcium delivered through separate tablets to the iron absorption from a regular supplement that combined iron and calcium in 1 tablet.

METHODS

Twelve healthy non-pregnant women of child-bearing age were recruited to participate in the study. Each woman signed a written consent form after reviewing the protocol, which was approved by the Ethics Review Board at The Hospital for Sick Children (REB# 100000127). After a night fast, the women were randomized to receive either 1 tablet of low iron (LI) without calcium (PregVit a.m.) or 1 tablet of high iron and calcium (HICA) (Materna) in a crossover methodology. The PregVit p.m. tablet, typically taken in the evening, does not contain iron, and was not tested in the recent study. See Table 1 for

Table 1. Composition of Two Prenatal Supplements

Component	PregVit* (a.m. & p.m. tablets)		Materna (1 tablet)
Vitamin A	2700 IU (β-carotene)	a.m.	1500 IU (β-carotene) 1500 IU (acetate)
Vitamin B ₁ (thiamin)	3 mg	a.m.	3 mg
Vitamin B ₂ (riboflavin)	3.4 mg	a.m.	3.4 mg
Vitamin B ₆	10 mg	a.m.	10 mg
Vitamin C	120 mg	a.m.	100 mg
Vitamin E	30 IU	a.m.	30 IU
Copper	2 mg	a.m.	2 mg
Iodine	0.15 mg	a.m.	0.15 mg
Iron	35 mg	a.m.	60 mg
Magnesium	50 mg	a.m.	50 mg
Niacinamide	20 mg	a.m.	20 mg
Pantothenic acid (calcium pantothenate)	5 mg	a.m.	10 mg
Zinc	15 mg	a.m.	25 mg
Vitamin B12 (cyanocobalamin)	12 µg	p.m.	12 µg
Vitamin D (cholecalciferol)	250 IU	p.m.	250 IU
Calcium	300 mg	p.m.	250 mg
Folic acid	1.1 mg	p.m.	1 mg
Biotin	0		30 µg
Chromium	0		25 µg
Manganese	0		5 mg
Molybdenum	0		25 µg
Selenium	0		25 µg

*In this study only the a.m. tablet, containing iron, was given to the participating women.

the composition of both supplements. Through an indwelling catheter, a 5 mL blood sample was obtained at 8:00 a.m. to measure the baseline serum iron level. The women then received 1 of the multivitamins, and additional 5 mL blood samples were drawn at 1, 2, 3, 4, 6, and 8 hours after they ingested the tablet. At 4 hours after taking the study dose, the women ate their delayed breakfast: a standardized meal of 2 white rolls with butter, 1 scrambled egg, and a non-carbonated, non-caffeinated beverage providing 2.4 mg of iron (Table 2). The women repeated the procedure in a crossover design, receiving the other multivitamin on a different day. The study days were scheduled so that the tests were conducted on the same day of the women's menstrual periods to control for varying iron levels.²

The blood samples were collected in Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ), allowed to clot at room temperature for 30 minutes, and then centrifuged at 1500 rpm for 15 minutes at 4°C. The serum was separated and immediately stored at -20°C. All samples were analyzed as a batch within 2 months, to keep the analytical error constant. Serum iron samples were measured using a Synchron LX-20 (Beckman Coulter, Inc., Fullerton, CA). The coefficient of variation of the method was 1.9% to 2.2%. The mean serum iron values for area under the concentration-time curve (AUC) of iron was calculated using the trapezoid rule,²³ and compared for the 2 tablets by the paired Student *t* test. In addition,

Table 2. Iron Content of the Standardized Meal

Item	Iron Content (mg)
1 large egg	0.6
2 white rolls	1.8
1 pat of butter	trace
355 mL non-caffeinated beverage	0
Total	2.4

standard iron absorption per mg of iron was calculated as the ratio between the AUC achieved by each of the vitamin preparations and the iron dose, and compared using the same statistical test. With 12 women and based on the known variability of serum iron levels, a detection of a 20% difference in AUC with a power of 80% and an alpha of 5% was possible.²⁴

RESULTS

The mean age of the 12 women was 23.75 years (range, 18–32 years). One woman discontinued the study upon experiencing stress-induced gastric irritations 2 weeks after the first study date. The other women did not report any adverse effects from the single tablet administration. The mean group AUC value for serum iron was $79.1 \pm 36.0 \mu\text{M}\cdot\text{h}$ for LI and $91.4 \pm 50.4 \mu\text{M}\cdot\text{h}$ for HICA ($P = .37$; Figure 1). Upon standardizing the AUC for dose, the relative absorption over the 8-hour time period with LI was significantly higher ($2.3 \pm 1.0 \mu\text{M}\cdot\text{h}/\text{mg}$) than for HICA ($1.5 \pm 0.8 \mu\text{M}\cdot\text{h}/\text{mg}$) ($P = .021$; Figure 2).

DISCUSSION

In this study, serum iron levels were not different with LI and HICA, despite the HICA tablet having almost twice the amount of iron as the LI tablet. The standardized meal given 4 hours after the first dose and containing 2.4 mg of iron was chosen to minimize the amount of iron given, as well as to exclude any ingredients known to inhibit the absorption of iron. Because the time to peak (T_{max}) of iron in the prenatal supplements was 3 hours,^{24,25} the meal was given 4 hours after the administration of the prenatal supplement so that it would not interfere with the peak iron plasma concentrations.^{24,25} Overall absorption of iron was calculated from the AUC, which is

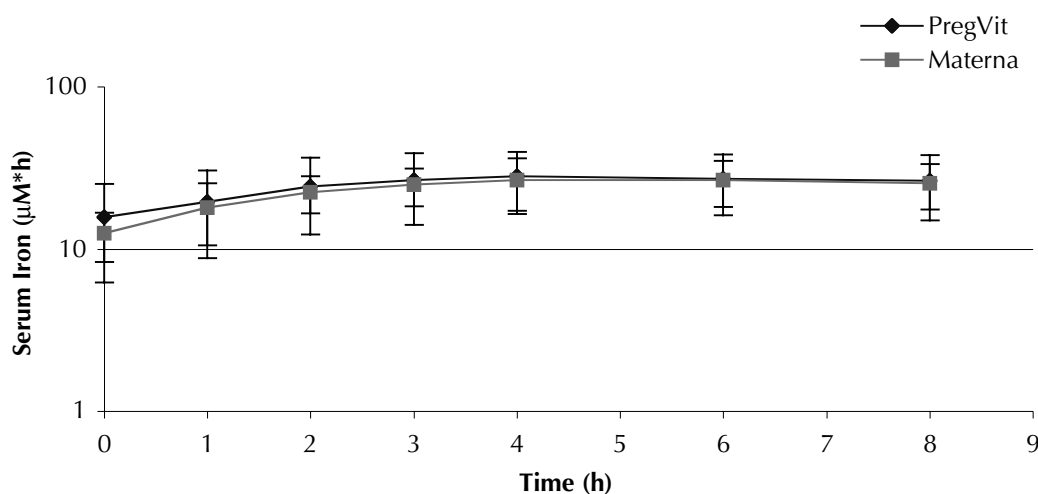
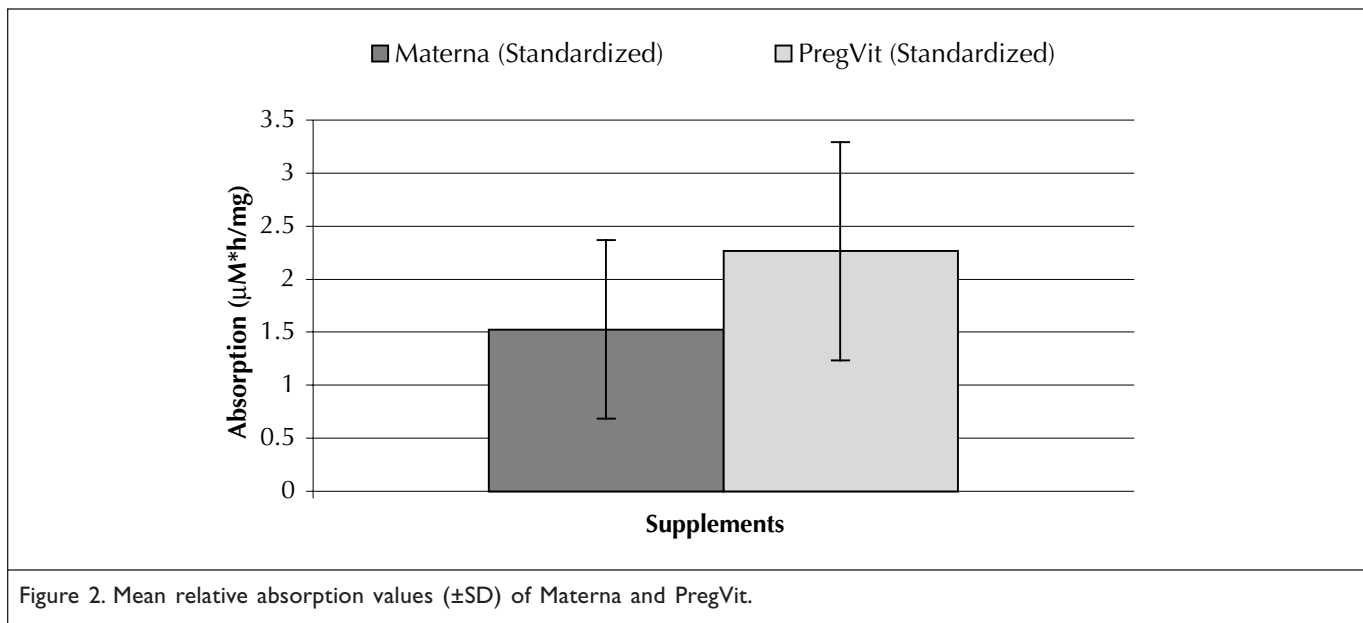


Figure 1. Mean AUC values (\pm SD) of Materna and PregVit.



superior to single-level measurements, as it gives the integrated change in levels.

The relative absorption of iron was significantly higher for LI, suggesting that the separation of iron administration from calcium allowed improved bioavailability. Calcium inhibition of iron is partially dose-dependent, with a threshold of 40 mg calcium and maximum inhibition at 300 mg of calcium.²⁶ Thus, the 250 mg of calcium found in the HICA supplement would likely result in close to the maximal inhibition of iron absorption from calcium of 50% to 60%.¹⁹ Our study implies that the calcium-iron interaction may have a substantial role in the iron absorption. In analyzing these results, it is important to discuss the fact that iron dose affects the extent of iron absorption.²⁷⁻³¹ Although studies have documented an inverse relationship between increased iron dose and percentage of iron absorbed,^{27,29} a study by Middleton *et al.*³² found that when administering iron supplements in doses of 96 mg, 140 mg, and 163 mg, iron AUC values increased in a linear fashion over a period of 7.5 hours. Hence, the differences between HICA (60 mg) and LI (35 mg) iron doses are well within the linear portion of the absorption curve. As a result, the AUC similarities between the 2 multivitamins cannot be explained by different extent of absorption due to dose discrepancies.

Other mineral interactions may have affected our results. For example, ascorbic acid is known to be a potent enhancer of iron absorption and can also overcome the effects of inhibitors, such as calcium, in a dose-related response.³³ The 120 mg of ascorbic acid (vitamin C) in the LI supplement was higher than the 100 mg ascorbic acid in the HICA supplement and substantially higher relative to the amount of iron (35 mg vs. 60 mg, respectively). The higher iron:ascorbic acid ratio of 1:3.4 in LI compared to the 1:1.6 ratio in HICA may have helped in absorption of the iron in the LI tablet. Furthermore, the HICA

supplement contains 10 mg more zinc, known to inhibit iron absorption, than the LI supplement, along with 5 mg of manganese sulfate, which may also have inhibited iron absorption.³⁴

Based on our findings, LI may have a lower risk of iron complications and thus be an appropriate supplement for women who cannot tolerate the adverse effects associated with higher doses of iron. In particular, women experiencing nausea and vomiting of pregnancy (NVP) may be more prone to discontinue the daily use of their prenatal supplements, as the iron-associated side effects exacerbate their pre-existing condition. Indeed, in one study from our unit, of 196 women with NVP, 36% discontinued the use of HICA due to the iron-associated side effects.³⁵

To avoid variability arising from the many physiological changes that occur in pregnancy,³⁶ a special concern in a crossover study design in that pregnant women may have different physiological states, such as iron levels or body weight, after 1 month of study, we recruited non-pregnant women for this study.

During the first trimester of pregnancy, iron requirements are reduced due to the cessation of menstruation,¹ but they subsequently rise steadily.¹ Although there is evidence that the rate of iron absorption during the third trimester depends on maternal iron status,³⁷ new evidence suggests that part of the etiology of iron deficiency during pregnancy may be the reduction of iron utilization.³⁸ For a given level of iron stores, however, there is no evidence of different absorption rates during pregnancy.

CONCLUSION

Our study demonstrated that the absorption of iron from a low-iron-containing supplement that delivered iron separately from calcium was similar to that from a supplement with almost twice

the amount of iron. This was probably due to the exclusion of calcium from the a.m. tablet with LI, as well as lower amounts of other inhibitors of iron absorption. Furthermore, iron in the LI supplement had a higher relative bioavailability than iron in the HICA supplement. Further clinical studies are required to evaluate whether an LI supplement will indeed be better tolerated by women sensitive to iron-induced adverse effects.

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REFERENCES

- Bothwell TH. Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr* 2000;72:257S–64S.
- Bothwell TH, Conrad ME, Cook JD, Finch CA. Iron metabolism in man. Oxford: Blackwell Scientific Publications; 1979. p. 14.
- Nova Scotia Heart Health Program, Nova Scotia Department of Health and Health and Welfare Canada. Report of the Nova Scotia Nutrition Survey. Halifax (NS): Nova Scotia Department of Health; 1993.
- Bertrand L. Les Québécoises et les Québécois mangent-ils mieux? Rapport de l'enquête québécoise sur la nutrition, 1990. Montréal: Ministère de la Santé et des Services sociaux, Gouvernement du Québec; 1995.
- O'Connor DL. The folate status of Canadian women. In: Koren G, editor: Folic acid for the prevention of neural tube defects. Toronto: The Motherisk Program; 1995. p. 73–87.
- McCourt C. Folic acid and neural tube defects: policy development in the Department of Health Canada. In: Koren G, editor: Folic acid for the prevention of neural tube defects. Toronto: The Motherisk Program; 1995. p. 95–101.
- Turgeon-O'Brien H, Larocque I, Desmeules C. Depletion des réserves en fer chez un groupe d'adolescentes et de femmes enceintes de la région de Québec. *Journal du Praticien, Revue Médicale de Liège* 1994;140:529.
- Savoie N. Impact of maternal anemia on the infant's iron status at 9 months of age. *Can J Public Health* 2002;93(3):203–7.
- Rioux MF, Michaud J. Maternal anemia in the southeast and northeast regions of New Brunswick and the impact on hematological parameters and the growth of the newborn [Maternelle dans les régions du sud-est et nord-est du Nouveau-Brunswick et impact sur les paramètres hématologiques et la croissance du nouveau-né]. *Can J Diet Pract Res* 2001;62(2):70–5. French.
- Turgeon-O'Brien H, Santure M, Maziade J. The association of low and high ferritin levels and anemia with pregnancy outcome. *Can J Diet Pract Res* 2000;61:121–7.
- Hodgins S, Dewailly E, Chatwood S, Bruneau S, Bernier F. Iron-deficiency anemia in Nunavik: pregnancy and infancy. *Int J Circumpolar Health* 1998;57(Suppl):135–40.
- Beaton GH. Iron needs during pregnancy: do we need to rethink our targets? *Am J Clin Nutr* 2000;72:265S–71S.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academy Press; 2002. p. 770–3.
- Frykman E, Bystrom M, Jansson U, Edberg A, Hansen T. Side effects of iron supplements in blood donors: superior tolerance of heme iron. *J Lab Clin Med* 1994;123:561–4.
- Galloway R, McGuire J. Determinants of compliance with iron supplementation: supplies, side effects, or psychology? *Soc Sci Med* 1994;39:381–90.
- Sifakis S, Pharmakides G. Anemia in pregnancy. *Ann NY Acad Sci* 2000;900:125–36.
- Alward N, Kevany J. Iron supplementation during pregnancy: a survey of the current situation. *Ir J Med* 1984;77:112–4.
- Committee on Nutritional Status during Pregnancy and Lactation, Food and Nutrition Board. Nutrition During Pregnancy. Washington (DC): National Academy of Sciences; 1990. p. 292.
- Bendich A. Calcium supplementation and iron status of females. *Nutrition* 2001;17:46–51.
- Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. *Am J Clin Nutr* 1991;53:106–11.
- Zijp IM, Korver O, Tijburg LB. Effect of tea and other dietary factors on iron absorption. *Crit Rev Food Sci Nutr* 2000;40:371–98.
- Office of Nutrition Policy and Promotion, Health Products and Food Branch, Health Canada. Nutrition for a healthy pregnancy: national guidelines for the childbearing years. Ottawa: Health Canada; 2002. Available at <www.hc-sc.gc.ca/hpfb-dgpsa/onpp-bppn/national_guidelines_06_e.html#back54.2004>. Accessed December 19, 2003.
- Rowland M, Tozer T. Clinical pharmacokinetics: concepts and applications. Baltimore (MD): Williams & Wilkins; 1995. p. 469–71.
- Dawson EB, Evans DR, McGanity WJ, Conway ME, Harrison DD, Torres-Cantu FM. Bioavailability of iron in two prenatal multivitamin/multimineral supplements. *J Reprod Med* 2000;45:403–9.
- Rubel HR, Giep NN. Effect of food on the availability of iron from three multivitamin/mineral supplements during pregnancy. *Clin Ther* 1987;9:311–7.
- Hallberg L. Does calcium interfere with iron absorption? *Am J Clin Nutr* 1998;68:3–4.
- Bothwell TH, Pirzio-Biroli G, Finch CA. Iron absorption: factors influencing absorption. *N Engl J Med* 1958;51:24–36.
- Harju E. Clinical pharmacokinetics of iron preparations. *Clin Pharmacokinetics* 1989;17:69–89.
- Smith MD, Pannacciulli IM. Absorption of inorganic iron from graded doses: its significance in relation to iron absorption tests and the 'mucosal block' theory. *Br J Haematol* 1958;4:428–34.
- Wessling-Resnick M. Iron transport. *Annu Rev Nutr* 2000;20:129–51.
- Uzel C, Conrad ME. Absorption of heme iron. *Semin Hematol* 1998;35:27–34.
- Middleton EJ, Nagy E, Morrison AB. Studies on the absorption of orally administered iron from sustained-release preparations. *N Engl J Med* 1966;274:136–9.
- Bothwell TH. Overview and mechanisms of iron regulation. *Nutr Rev* 1995;53:237–45.
- Rossander-Hulten L, Brune M, Sandstrom B, Lonnnerdal B, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. *Am J Clin Nutr* 1991;54:152–6.
- Ahn E, Koren G. The effects of nausea and vomiting of pregnancy on usage of Materna. *Can J Clin Pharmacol* 2003;10:150.
- Loebstein R, Lalkin A, Koren G. Pharmacokinetic changes during pregnancy and their clinical relevance. In: Koren G, editor: Maternal-fetal toxicology. Toronto (ON): Marcel Dekker, Inc.; 2001. p. 1–21.
- O'Brien KO, Zavaleta N, Abrams SA, Caulfield LE. Maternal iron status influences iron transfer to the fetus during the third trimester of pregnancy. *Am J Clin Nutr* 2003;77:924–30.
- Whittaker PG, Barrett JF, Lind T. The erythrocyte incorporation of absorbed non-haem iron in pregnant women. *Br J Nutr* 2001;86:323–9.