NOTE / NOTE

Oral administration of lactoferrin increases hemoglobin and total serum iron in pregnant women[1]

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Abstract: Iron deficiency anemia (IDA) during pregnancy continues to be of world-wide concern. IDA is a risk factor for preterm delivery and subsequent low birth weight, and possibly for poor neonatal health. Iron supplementation in pregnancy is a widely recommended practice, yet intervention programs have met with many controversies. In our study, 300 women at different trimesters of pregnancy were enrolled in a trial of oral administration of ferrous sulfate (520 mg once a day) or 30% iron-saturated bovine lactoferrin (bLf) (100 mg twice a day). Pregnant women refusing treatment represented the control group. In this group hemoglobin and total serum iron values measured after 30 d without treatment decreased significantly, especially in women at 18–31 weeks of pregnancy. In contrast, after 30 d of oral administration of bLf, hemoglobin and total serum iron values increased and to a greater extent than those observed in women treated orally for 30 d with ferrous sulfate, independently of the trimester of pregnancy. Unlike ferrous sulfate, bLf did not result in any side effects. These findings lead us to hypothesize that lactoferrin could influence iron homeostasis directly or through other proteins involved in iron transport out of the intestinal cells into the blood.

Key words: lactoferrin, anemia, hemoglobin, serum iron.

Résumé : L'anémie ferriprive (AF) durant la grossesse est une préoccupation mondiale continuelle. L'AF constitue un facteur de risque d'accouchement prématuré qui a pour conséquence un petit poids à la naissance et possiblement une mauvaise santé néonatale. Le supplément en fer durant la grossesse est une pratique largement recommandée quoique ces programmes d'interventions aient suscité la controverse. Lors de notre étude, 300 femmes parvenues à différents trimestres de grossesse ont été recrutées afin de recevoir du sulfate ferreux oral (520 mg une fois par jour), ou de la lactoferrine bovine saturée de fer à 30 % (Lfb) (100 mg deux fois par jour). Les femmes enceintes refusant le traitement ont constitué le groupe contrôle. Dans ce groupe, les valeurs d'hémoglobine et de fer sérique total mesurées après 30 jours sans traitement ont diminué, spécialement chez les femmes parvenues à leur troisième trimestre de grossesse. À l'opposé, après 30 jours d'administration de Lfb, les valeurs d'hémoglobine et de fer sérique total ont augmenté de façon plus importante que celles des femmes traitées pendant 30 jours par l'administration orale de sulfate ferreux, et ce, de façon indépendante du trimestre de grossesse. Contrairement au sulfate ferreux, l'administration de Lfb n'était accompagnée d'aucun effet secondaire. Ces résultats nous conduisent à poser l'hypothèse que la lactoferrine puisse influencer directement l'homéostasie du fer directement ou par l'intermédiaire d'autres protéines impliquées dans le transport du fer des cellules intestinales vers le sang.

Mots clés : lactoferrine, anémie, hémoglobine, fer sérique.

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Introduction

In humans, iron absorption occurs in the proximal small intestine (duodenum) in which 1–2 mg of dietary iron is absorbed daily, ensuring that there are iron supplies in the bone marrow. Therefore, iron homeostasis is regulated primarily at the site of iron absorption in the intestine in response to body iron requirements, as mammals lack a regulated pathway for iron excretion (Andrews 2000; Wessling-Resnick 2000; Frazer and Anderson 2003).

Iron deficiency (ID) is the most common nutritional deficiency, and iron deficiency anemia (IDA) is the most frequent anemia in the world (Umbreit 2005). Pregnant women can be at risk of ID and IDA because of the extra iron required by the growing fetus, placenta, and increased maternal red cell mass (Makrides et al. 2003). Values of hemoglobin corresponding to 11 g/dL or less and total serum iron corresponding to 30 mg/dL or less define ID and IDA, respectively. Iron supplementation is generally recommended during pregnancy to counteract ID and IDA, and thus avoid an increased risk of preterm birth (Scholl 2005).

There are 3 possible ways to prevent and control the development of ID and IDA. These encompass dietary diversification, food fortification, and individual supplementation (Zlotkin 2002). The preferred treatment of these pathologies consists of oral administration of iron as ferrous sulfate. However, the large quantity of ferrous sulfate to be administered to patients with ID and IDA is related to the poor bioavailability of inorganic iron. Moreover, oral administration of ferrous sulfate causes many problems, including gastrointestinal discomfort, nausea, vomiting, diarrhea, and constipation, and it may sometimes increase the susceptibility to infections.

The idea of orally administering lactoferrin as an iron-supplying molecule is very appealing, even if to date the studies performed have shown conflicting results, reporting either enhancement or inhibition of intestinal iron delivery (Tsuji et al. 2001; Brock 2002; Hernell and Lonnerdal 2002; Ward and Conneely 2004).

The specific aim of our study was to verify whether oral administration of bovine lactoferrin (bLf) could be an alternative to ferrous sulfate in pregnant women suffering from ID or IDA. Here we report the results of a clinical trial involving 300 women enrolled at different trimesters of pregnancy who had ID or IDA. These women were divided into 3 groups: the first included women refusing treatment (control group), the second was women treated orally with ferrous sulfate, and the third was women treated orally with bLf.

The results showed that, independently of the trimester of pregnancy, the oral administration of bLf resulted in an increase of hemoglobin and total serum iron values, as compared with levels observed in women treated with ferrous sulfate or untreated women.

Materials and methods

Treatment

Lactoferrin

Bovine lactoferrin was given orally as Lf100 (Dicofarm, Rome, Italy). Each capsule of Lf100 contained 100 mg of bLf. The Italian standard treatment consists of 1 capsule twice a day before meals. The purity of bLf was checked by SDS–PAGE stained with silver nitrate, and its iron saturation corresponded to about 30%, as determined by optical spectroscopy.

Ferrous sulfate

Ferrous sulfate was administered orally as Ferro-Grad (Abbott Laboratories, USA). One tablet contained 520 mg of ferrous sulfate. The Italian recommended treatment consists of 1 tablet once a day.

Pregnant women

Pregnant women were recruited from the obstetrical patients seen at S. Andrea Hospital, Department of Obstetrics and Gynecology of the University of Rome "La Sapienza". A total of 300 pregnant women, at different ages, parities, and trimesters of pregnancy, suffering from ID or IDA were included in this clinical trial. The pregnant women who refused treatment with oral iron supplementation represented the control group. The remaining subjects were randomly divided into 2 groups. The first group received the Italian standard treatment, according to hematological consensus, of 520 mg of ferrous sulfate administered orally once a day, and the second group received 1 capsule of Lf100 administered orally twice a day. The dose of 520 mg ferrous sulfate corresponded to 156 mg elemental iron supplemented per day, and that of 200 mg bLf/day corresponded to 8.8 mg of ferric ions.

Laboratory tests

Hemoglobin and total serum iron levels were assessed from venous blood as previously described (Meier et al. 2003). Laboratory tests were performed upon enrollment (time 0) in the prenatal program and after 30 d. The women tested were at different stages of pregnancy, classified as follows: from 12 to 17, from 18 to 23, and from 24 to 31 weeks.

Statistical analysis

Statistical analysis was performed using the Student's t test for unpaired data. P values < 0.01 were considered significant.

Results

Over a study period of 12 months, 300 subjects with ID or IDA were enrolled in a clinical trial. Thirty-one subjects were ultimately excluded or lost to analysis, 4 moved or were lost to follow-up after 30 d of oral iron supplementation, 3 had spontaneous abortions, and 3 were excluded for other reasons.

Of the 259 pregnant women participating, 54 refused oral iron supplementation (control group), 98 were treated with oral administration of 520 mg ferrous sulfate once a day, and 107 with 100 mg bLf twice a day.

For all women, the hemoglobin and total serum iron levels are reported as range and median values at the time of enrolment (time 0) and after 30 d (Table 1). In women refusing treatment, the lack of iron supplementation did not significantly influence the hemoglobin and total serum iron values for those at 12–17 weeks of pregnancy, whereas a

Table 1. Hemoglobin and total serum iron values in pregnant women enrolled in a clinical trial of iron supplementation.

				Time 0, median value (range)		After 30 d, median value (range)	
Subjects	Week of pregnancy	No. of subjects	Age (years)	Hemoglobin (g/dL)	Total serum iron (μg/dL)	Hemoglobin (g/dL)	Total serum iron (μg/dL)
Refusing treatment (control group)	12–17	20	25–30	11.2 (11.0–11.4)	40.0 (33–47)	11.2 (11.0–11.5)	35.0 (30–40)
	18–23	20	28–32	11.2 (10.7–11.8)	40.0 (33–47)	10.6 (10.5–10.8)	26.5 (25–28)
	24–31	14	24–30	11.0 (10.9–11.2)	36.5 (30–43)	10.2 (10.0–10.4)	23.0 (20–26)
Receiving ferrous sulfate treatment	12–17	31	31–39	10.3 (10.0–10.7)	38.5 (33–44)	11.2 (11.0–11.5)	52.5 (45–60)
	18–23	32	30–33	10.8 (10.2–11.4)	40.0 (33–47)	11.9 (11.9–12.0)	63.0 (58–68)
	24–31	35	28–39	11.2 (10.7–11.8)	52.0 (48–56)	11.9 (11.5–12.3)	59.0 (48–70)
Receiving bLf treatment	12–17	38	25–37	11.0 (10.1–11.9)	48.5 (33–64)	12.8 (12.3–13.5)	110.0 (80–40)
	18–23	27	31–39	11.2 (10.7–11.8)	44.5 (25–64)	12.5 (12.0–13.0)	93.0 (70–116)
	24–31	42	28–37	11.1 (10.6–11.7)	46.5 (27–65)	12.8 (12.8–13.0)	97.5 (85–110)

Table 2. Delta mean values for hemoglobin and total serum iron in a clinical trial of iron supplementation.

	Delta mean values (range)	
Subjects	Hemoglobin (g/dL)	Total serum iron (μg/dL)
(*a*) Refusing treatment (control group)	–0.4 (0 to –0.8)	–10.2 (–0.5 to –15.5)
(*b*) Receiving ferrous sulfate treatment	0.9 (0.7 to 1.1)	8.0 (10.5 to 12.5)
(*c*) Receiving bLf treatment	1.5 (1.3 to 1.8)	54.2 (46.5 to 65.5)
***P* value**		
(*a*) versus (*b*)	<0.01	<0.01
(*a*) versus (*c*)	<0.01	<0.01
(*b*) versus (*c*)	0.02	<0.01

significant decrease of hemoglobin and total serum iron values was observed in women at 18–31 weeks of pregnancy ($P < 0.01$). In all treated women, the hemoglobin and total serum iron values showed a significant increase ($P < 0.01$) after 30 d of treatment, independently of duration of pregnancy. However, in pregnant women receiving ferrous sulfate, the increase in mean values of hemoglobin and total serum iron (0.9 g/dL and of 8.0 μg/dL, respectively) were lower than those observed in women receiving bLf (1.5 g/dL and 54.2 μg/dL, respectively).

To evaluate the significance of the data obtained, delta values were calculated by subtracting the median values of hemoglobin and total serum iron at time 0 and after 30 d, with or without treatment (Table 2). Delta values of hemoglobin and total serum iron in pregnant women treated with ferrous sulfate or bLf were significantly higher than those observed in the untreated pregnant women (Table 2). The delta value of total serum iron obtained in the bLf-treated group as compared with that of the ferrous-sulfate-treated group was also significantly higher, but that of hemoglobin failed to reach significance (Table 2).

With respect to side effects, of 98 women receiving ferrous sulfate, 95% had stomach pain, cramps, and constipation, and 2% had at least 1 diarrhea episode. By contrast, no side effects were observed in the 107 women given bLf.

Conclusions

Our results demonstrate that in pregnant women with ID or IDA receiving iron supplementation, hemoglobin and total serum iron values measured after 30 d of treatment were higher than those observed in pregnant women refusing treatment, independently of the duration of pregnancy. The women given bLf orally for 30 d showed higher hemoglobin values than those observed in women receiving ferrous sulfate orally for 30 d, even though this increase failed to reach significance ($P = 0.02$). Total serum iron values, however, were significantly higher in bLf-treated women than in ferrous-sulfate-treated ones ($P < 0.01$), despite the fact that the amount of iron supplied by bLf (8.8 mg/d) was lower than that supplied by ferrous sulfate (156 mg/d).

This is the first in vivo study of pregnant women with ID or IDA reporting that the oral administration of partially iron-saturated bLf enhances intestinal iron delivery better than ferrous sulfate. Interestingly, the absence of side effects of bLf oral administration resulted in very high compliance among treated women.

This significantly high increase of total serum iron in all bLf-treated women leads us to suppose that lactoferrin could influence iron homeostasis. It is likely that bLf could act not only by directly supplying iron to intestinal epithelial cells through a specific lactoferrin-binding receptor (Suzuki et al. 2005) but also by a more complex mechanism of modulation of other proteins involved in iron transport out of the intestinal cells into the blood (Fleming and Bacon 2005).

Taken together, the data encourage us to carry out other clinical trials with pregnant women and also with gynecological patients with ID or IDA.

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