Importance of Pre-Pregnancy and Pregnancy Iron Status: Can Long-Term Weekly Preventive Iron and Folic Acid Supplementation Achieve Desirable and Safe Status?

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Most women worldwide enter pregnancy without adequate iron reserves or are already iron deficient. Estimates of iron needs during pregnancy are markedly reduced when iron reserves are available. The needs of absorbed iron to correct mild to moderate anemia in the last two trimesters are estimated. Pre-pregnancy and prenatal weekly supplementation can improve iron reserves effectively and safely, preventing excess iron and favoring better pregnancy outcomes. We explain how the weekly supplementation idea was developed, why current hemoglobin norms may be inadequately high (especially in pregnancy), and why excess iron as recommended by many agencies for developing populations can be undesirable.

Key words: anemia, iron status, women of reproductive age, iron deficiency.

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IRON STATUS OF WOMEN OF REPRODUCTIVE AGE

Women of reproductive age (WRA) are at risk of developing iron deficiency, which if continued for several months can progress to anemia. The World Health Organization (WHO) estimates that 43% of all non-pregnant women 15 to 59 years of age who live in the developing world have anemia; during pregnancy, this prevalence increases to 56%. These percentages translate to 535 and 54 million women, respectively, 41% of whom reside in southeast Asia.[1] Mason et al.[2] gave prevalence percentages for women 15 to 49 years of age for the year 1990 as 26.5% for non-pregnant women and 32.2% for pregnant women; for 2000, the numbers were 31% for non-pregnant women and 30.5% for pregnant women.

WRA and pregnant women are also at risk of anemia in many industrialized countries. Representative data from the United States indicate that 5% of non-pregnant women are anemic, that the prevalence of anemia increases to 17% among pregnant women, and that the prevalence is as high as 33% among pregnant women of low socioeconomic groups.[3,4] Furthermore, iron deficiency among white, non-pregnant women in the United States is reported to be 10%, while it is 19% among African Americans and 22% among Mexican Americans. It is distressing that prevalence rates have increased by 2% to 4% from the period 1984–1988 to the period 1999–2000, despite fortification of flour with iron.[3]

In their study at the University of California at Berkeley, Viteri et al.[5] reported that 9% of non-pregnant female students presented with anemia and responded to iron administration, while 20% presented with depleted iron stores as defined by serum ferritin under 15 μg/L. The proportion of iron deficiency to anemia in the student population was 2.2 to 1, indicating that for each woman with iron deficiency anemia, there were between 2 and 2.5 who did not fit the criteria for being classified as anemic. A similar ratio of iron deficiency to iron deficiency anemia has been reported in many studies accepted by WHO and the US Centers for Disease Control and Prevention (CDC).[6] Unfortunately, data about women in the developing world that would allow estimates of the magnitude of iron deficiency anemia independent from anemia due to other causes are lacking. However, most studies that have looked into these conditions confirm that more than 80% of cases of anemia in women, especially during pregnancy, are associated with or have an important iron deficiency component.[7-9]

Ideally, to meet iron needs during gestation, women should have 300 mg or more of iron reserves prior to conception.[10] Based on the information available, many

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women, even in the industrial world, enter pregnancy with little or no iron to mobilize during the second half of pregnancy, when the requirement for that essential mineral increases significantly.[11] Fifty-six percent of non-pregnant US WRA surveyed in The National Health and Nutrition Examination Survey in 1988–1994 (NHANES III) had less than 300 mg of iron reserves based on the serum transferrin receptor (TfR)/serum ferritin ratio for determining iron stores[12] (Table 1). A longitudinal study of non-anemic Mexican women (who received prenatal care but no iron supplementation unless they developed anemia) followed through pregnancy starting at gestational week 10 showed that the incidence of anemia as pregnancy progressed became higher (nearly five times greater near term) among those with serum ferritin levels under 20 μg/L compared with those over 20 μg/L prior to conception.[13]

In China, birth weight and preconceptional hemoglobin (Hb) were shown to be directly correlated.[14] There are few accurate data on the distribution of estimated iron reserves in women in developing countries. In a study comparing serum ferritin levels in rural and urban Guatemalan non-pregnant women,[15] the effects of location and hookworm infestation were very evident in relationship to iron reserves (Table 1).

Most women throughout the world enter pregnancy with less than desirable iron reserves, many with depleted iron stores, and, by current criteria, with iron deficiency even when they are not anemic.

PRE-PREGNANCY IRON STATUS CAN BE IMPROVED WHERE IDEAL DIETS ARE UNAVAILABLE

Iron Fortification

Iron stores estimated by a proposed algorithm that considers several indicators of iron status[11] were obtained during a 3-year iron fortification study in the total population of non-pregnant women living in two coastal (tropical) and two highland Guatemalan towns.[16] The two coastal populations and one of the highland populations received iron-fortified sugar (mean intake 4 mg/d of iron) from NaFeEDTA, while the remaining highland community received unfortified sugar and served as a control. Table 2 presents the distributions of iron stores for the women in each community before and after 3 years of fortification. The effectiveness in improving iron status above 300 mg in the communities receiving iron-fortified sugar with NaFeEDTA is evident. Even though the increase occurred mostly in the first year, by the third year, iron reserves were still slowly increasing (by about 40 mg/year).

A recent randomized, controlled study[17] from Vietnam among moderately anemic women reported changes in iron stores and correction of iron-deficiency anemia after 6 months of consuming fish sauce fortified with NaFeEDTA providing 10 mg of iron daily. At baseline, 70% of the anemic women were iron deficient and had mean iron stores of about 15 mg. At the end of the study, mean iron stores were about 186 mg, the highest value attained being about 250 mg.

Another study in Vietnam evaluated the impact of uncontrolled consumption of NaFeEDTA-fortified fish sauce (providing 0.5 mL iron/mL fish sauce) provided free of charge in a sample of Vietnamese women, 25% of whom were anemic. After 12 months of intervention, less than 5.5% of the women had iron deficiency based on ferritin concentrations. This indicator of iron reserves had increased an average of 70 μg/L, translating to median iron stores of 350 mg.[18]

These studies demonstrate the effectiveness of food fortification by the addition of iron in a safe compound with good iron bioavailability when added to diets that do not favor iron absorption, even in women with mild hookworm infestation.

Preventive Supplementation

Centralized industrial production of fortified foods may not reach all populations, particularly the target populations most vulnerable to iron deficiency: infants, chil-

Table 1. Percent Distribution of Women of Reproductive Age by Iron Stores in the United States[13] and in Different Locations in Guatemala[14]*

Population	Iron Stores (mg)						
	≤0†	≤50	51–100	101–150	151–200	201–300	>300
United States	12%	2%	8%	9%	8%	17%	44%
Guatemala urban highlands (no hookworm)	39%	19%	15%	12%	4%	11%	0%
Guatemala rural highlands (no hookworm)	55%	17%	14%	7%	6%	1%	0%
Guatemala rural lowlands (hookworm positive)	67%	5%	4%	10%	6%	8%	0%

*Iron stores in the Guatemalan population were calculated only on the basis of serum ferritin values considering no reserves if plasma ferritin fell below 15 μg/L (11) and 8 mg of iron stores/μg/L of ferritin if above 15 μg/L. Subjects were healthy, nulliparous women not using any fertility control methods and having normal serum folates.

†Reserves lower than 0 mean iron deficiency.

Table 2. Percent Distribution of Women of Reproductive Age by Iron Stores in Guatemala Before and After 3 Years of Consuming Iron-Fortified Sugar

Population	Iron Stores (mg)						
	≤0*	≤50	51–100	101–150	151–200	201–300	>300
Rural (severe hookworm infection)	61%/46%†	8%/8%	8%/7%	7%/6%	8%/5%	8%/10%	0%/18%
Rural (mild hookworm infection)	51%/40%	4%/10%	4%/6%	6%/7%	6%/4%	15%/10%	14%/23%
Rural (no hookworm infection)	48%/38%	7%/7%	4%/6%	6%/5%	3%/2%	6%/6%	26%/36%
Rural (no hookworm infection) controls	43%/47%	8%/5%	4%/4%	5%/4%	3%/5%	6%/5%	31%/30%

*Stores lower than 0 mean iron deficiency.
†Mean iron stores (mg) before/after 3 years.

dren, and WRA, especially those who are pregnant or prone to become pregnant. Several alternative approaches have demonstrated effectiveness: preventive weekly iron and folic acid supplementation[19]; food complements such as Sprinkles,[20] which contain iron and other essential nutrients and are added to foods at home; and supplements taken independently of food, such as foodlets.[21,22] To our knowledge, these last approaches have been evaluated only in infants and children and should not be considered here. However, we look forward to future studies with these same approaches directed at WRA. Among female college students in the United States, anemia was corrected in less than 3 months and iron stores improved after 7 months of weekly supplementation with 60 mg of iron and 250 μg of folic acid.[5]

Several studies based on weekly iron-folic acid supplementation in non-pregnant women,[5,23,24] as well as other studies presented in this supplement,[25-27] have indicated that iron reserves can be improved and anemia can be markedly reduced or totally corrected by this approach, particularly if the intervention is long term. Moreover, the superiority of weekly supplementation has been demonstrated in terms of efficiency and safety compared with administering daily iron supplements for 2 to 4 months per year, as has been suggested previously.[28] The objective of preventive weekly supplementation to improve iron reserves, reduce anemia, and reduce the risk of neural tube defects from folate deficiency is lost if iron-folic acid supplements are only administered in periodic courses of 4 months duration aimed at reducing anemia; this approach then becomes therapeutic rather than preventive.

The importance of adequate iron nutrition throughout life for optimal function is well known,[29] but the possible benefits of pre-pregnancy iron reserves on the course of pregnancy and its outcome is documented by only a few studies.[13,14,29] A study by Scholl et al.[30] illustrates the importance of taking multivitamin/mineral supplements containing iron prior to pregnancy and in the first trimester, which results in the reduction of low birth weight and very preterm deliveries and confirms the preventive effect on those outcomes by eliminating iron deficiency anemia early in pregnancy.

BRIEF HISTORY OF WEEKLY SUPPLEMENTATION WITH IRON AND FOLIC ACID

The idea of weekly iron supplementation was conceived as a preventive rather than a therapeutic measure for iron deficiency and its progression to anemia. More than 40 years of iron supplementation programs aimed at controlling gestational anemia have been ineffective. Therefore, the opportunity was ripe for exploring new possibilities for iron supplementation with a preventive approach. This approach considers the capacity of fresh intestinal cells to absorb iron and deliver it to transferrin in response to internal signals reflecting total body iron status and homeostatic need. Questions arose on the logic of daily oral administration of iron doses that far exceed the capacity of an individual to assimilate (absorb, utilize, and metabolize) iron safely. Is it inconsequential to maintain the intestinal mucosa and intestinal lumen with large amounts of unassimilated "reactive" iron? For many years, national and international agencies have proposed high daily doses of iron (60, 120, 240, and even ≥300 mg) for populations in whom the prevalence of gestational iron deficiency anemia is above 40%.[28,31,32] Estimates of the level of iron assimilated when supplemental iron doses above requirements are ingested for several days or weeks vary widely, from 2 to 3 mg/d to as high as 20 mg/d or even higher, depending on the dose and the total body iron status. In general, a sustained percentage of assimilated iron is less than 10% and bone marrow activity rarely increases more than three times the normal turnover.[33]

Studies in rats administered daily the equivalent of 120 mg of iron given to humans demonstrated that supplemental iron absorption by iron-deficient rats declined very rapidly, from 20% to 5%–6%, resulting in their intestinal contents and mucosa becoming overloaded with iron.[34,35] Daily supplemented iron-deficient rats also appeared to have impaired regulation of iron metabolism, causing significantly higher liver iron than normal, possibly by mass-effect-induced iron overload.[34] This undesirable condition was associated with severe oxidative stress, which was most evident in previously iron-deficient rats, but also manifested

in previously iron-normal, iron-supplemented rats.[35-38] In humans, elevation of "free" reactive iron in plasma and high ethane production occurred after ingestion of daily iron at recommended supplementary doses.[39,40] The apparent impaired regulation of iron metabolism and increased oxidative stress in previously iron-deficient rats strengthens the importance of preventing iron deficiency rather than correcting it once present.

The non-physiological conditions that occur when the recommended doses of daily iron supplements are given to already iron-deficient or even to normal animals and humans should be avoided. One option is to reduce the daily dose to levels that would be mostly absorbed and thus prevent the constant physiological disruption of the intestinal mucosa and metabolism. Another option could be to administer iron only in synchrony with the intestinal mucosal turnover that occurs every 3 days in the rat and every 5 to 6 days in the human.

The second option, that of presenting iron only to new mucosal cells, seemed most attractive for the reasons stated above, particularly if the objective was to prevent iron deficiency. The model tested in rats demonstrated that iron assimilation declined more slowly and was more than 2.5 times more efficient than when the same dose was administered daily.[34,35] Thus, the concept of weekly iron-folic acid supplementation as a public health approach to prevent iron deficiency and anemia was presented at the 1993 World Health Organization (WHO)/ UNICEF/United Nations University (UNU) meeting, the report of which was published in 2001.[32] If effective, weekly supplementation would also have additional operational advantages, including the need to distribute one-seventh the number of iron tablets, making the program easier for communities to manage. Furthermore, if side effects occurred at similar rates as after taking each daily dose, they would only occur once weekly rather than daily.[41]

In the last decade, many studies in different populations have confirmed that a weekly dose of iron could be effectively used for the prevention of iron deficiency in populations at risk, including during gestation. Even in short-term studies, this approach improves iron reserves and corrects mild to moderate anemia.[19,49] Unfortunately, in many cases, the therapeutic concept of correction of anemia has prevailed in the experimental designs comparing daily and weekly iron supplementation. However, even under these "unfavorable" conditions, the overwhelming majority of studies in different parts of the world with populations of different ages and sex have shown that the weekly approach is efficacious and effective.[49] The main reason that iron absorption studies in humans are conflicting is because they have been short term and conducted in essentially iron-normal populations.[50-53] Longer-term absorption in iron-deficient populations demonstrates a more efficient, sustained absorption than that from weekly supplementation.[54]

THE POSSIBLE IMPACT OF PRE-PREGNANCY IRON NUTRITION ON IRON STATUS DURING PREGNANCY

To illustrate the possible impact of two variables (pre-pregnancy iron reserves and iron supplementation) on the needs for absorbed iron to prevent iron deficiency during pregnancy, we constructed estimates of iron demands (in total milligrams) for well-nourished, non-

Table 3. Iron Needs (mg) during Pregnancy in the Absence and Presence of Pre-Pregnancy Iron Reserves and Gestational Anemia[42#]

Iron Supplemented	NO	YES(60 mg/d)
Basal body loss	216	216
Fetal accumulation	270	270
Placenta	80	80
Maternal Hb and tissue increments	322	400*
TOTAL	888	966†
Dietary Contribution‡	342	205§
Iron Deficit (mostly in the last two trimesters)‖		
No Iron Reserves	546 (888 − 342)	0¶
Using 300 mg Iron Reserves	246 (888 − 342 − 300)	0

*Iron supplemented increment in hemoglobin (Hb)[43]

†Delivery blood loss not considered

‡Assuming 0.8 mg and 1.5 mg of absorbed iron in first and last two trimesters, respectively[42]

§Assuming a conservative 40% reduction in food iron absorption in the supplemented group[44,45]

‖In theory, and based on the above assumptions, this amount of absorbed iron would be all that is needed from iron supplements to maintain iron balance during pregnancy; this could be achieved by absorbing 3 mg of iron/d in the last two trimesters using no reserves

¶60 mg of iron daily should not allow an iron deficit, and only 7% of the 60 mg supplement needs to be absorbed (966 mg − 205 mg) = 761 mg/(60 mg × 180 days = 10,800 mg) = 0.07, or 7%, under the above assumptions

#Note that if more food iron is absorbed late in pregnancy,[46] less iron absorbed from the supplements is needed)

anemic women (Table 3). These estimates assume an absorption of 0.8 mg of dietary iron in the first trimester of pregnancy and 1.5 mg in the last two trimesters,[42] either utilizing no iron from stores or using all of 300 mg of pre-pregnancy iron reserves. The milligrams of iron needed to be utilized, in the absence of anemia, from the intake of 60 mg of iron daily as a supplement during the last two gestational trimesters is also estimated. Table 4 expresses the estimates of iron deficits if mild to moderate anemia were present near term, as well as the amounts of iron needed to be absorbed during the last two trimesters of pregnancy to bring Hb concentrations to a non-anemic condition (110 g/L).

An adequate response to absorbed iron produces an Hb increment of 10 g/L per month. Therefore, for an Hb of 100 g/L, it would take 1 month to bring the Hb level to 110 g/L and 2 months to bring the Hb of 90 g/L to the desired 110 g/L level. This amounts to 7.1 mg/d for 30 days, for an intial Hb of 100 g/L and 7.0 mg/d for 60 days for an initial Hb of 90 g/L. The amounts of iron needed to be absorbed in the last 180 days (two trimesters) under these assumptions is:

- For an Hb of 100 g/L: 7.1 mg/d + 3.0 mg/d = 10.1 mg/d for 1 month (to replenish Hb deficit and satisfy basal needs) and 3.0 mg/d for 1 month (to satisfy basal needs), for a total of 483 mg.
- For an Hb of 90 g/L: 7.0 mg/d + 3.0 mg/d = 10.0 mg/d for 2 months (to replenish Hb deficit and satisfy basal needs) and 3.0 mg/d for 1 month (to satisfy basal needs), for a total of 690 mg.

These amounts of iron that need to be absorbed during the "anemia recovery" period constitute 16.8% of a 60 mg daily supplement for 1 month if Hb was 100 g/L, or 2 months if Hb was 90 g/L, respectively, under the assumed conditions. The times for recovery would vary if iron absorption from food and supplements were higher or lower.

Weekly supplementation with 120 mg of iron would cover the daily 3 mg deficit during the second and third trimesters (in the absence of iron reserves) if 17.4% of it were absorbed. It would take longer than 1 or 2 months to replenish the deficit in the case of anemia with Hb deficits of 10 and 20 g/L (Hb of 100 and 90 g/L, respectively), even if food iron absorption increased above the levels of 0.8 and 1.5 mg assumed in the calculations shown in Tables 3 and 4 and if the supplemental iron absorption values were also higher.

These calculations reveal the following:

1. Adequate pre-pregnancy iron reserves can be achieved by an adequate diet, food fortification, or preventive iron supplementation (low daily doses or weekly doses). Ideally, the last two types of intervention would include folate and, if needed, other nutrients such as vitamin A and zinc.
2. Correction of anemia during pregnancy is difficult and should therefore be prevented.

Table 4. Hematologic Parameters for a 60-kg Woman Pre-Pregnancy and Near Term when Non-Anemic and Near Term with Mild to Moderate Anemic Conditions (90 or 100 g Hb/L)

		Near Term			
				Mild to Moderate Anemia	
Parameter	Pre-Pregnancy Non-Anemic	Ideal Conditions	Minimal Non-Anemic	100 g/L	90 g/L
Total blood volume (TBV)	3645 mL[‡]	4964 mL	4,964 mL		
Total erythrocyte mass (TEM)	1677 mL[‡]	2012 mL[§]			
Total plasma volume (TPV)	1968 mL[‡]	2952 mL[§]			
Total hemoglobin mass (THbM)	466 g[‡]	559 g	499 g	453 g	408 g
Hemoglobin (Hb)	140 g/L	123 g/L	110 g/L		
Total Hb iron*	1584 mg	1901 mg	1697 mg	1540 mg	1389 mg
Iron deficit	0	0	0	157 mg	308 mg
Absorbed iron needed to correct deficit[†]				196 mg	385 mg

*THbM × 3.4 mg iron/g

[†]Assume 80% incorporation of absorbed iron into Hb[45]

[‡]TBV = 60.75 mL/g[47]; TEM = TBV × 46% (packed cell volume); TPV = 54% × TBV; THbM = TEM × 27.8 (mean corpuscular hemoglobin concentration)

[§]TEM is increased 20% from pre-pregnancy and TPV is increased by 50% from pre-pregnancy[48]

3. Weekly iron supplementation and its possible anemia-corrective capacity is valuable if sustained and started early in pregnancy.

One caveat to the calculations in Tables 3 and 4 may be the need to correct "anemia" of the magnitude considered in those tables, based on studies indicating that very adequate pregnancy outcomes occur when Hb is above 90 to 100 g/L.[56-62] Therefore, there is concern that the cutoff levels for gestational anemia proposed by the CDC and adopted by WHO might be too high,[32,63-65] as discussed below.

DEFINITIONS OF ANEMIA AND IRON DEFICIENCY AND THEIR CONSEQUENCES

Anemia can have two definitions: one from a public health perspective and the other from a functional perspective. The public health definition of anemia has been based on cutoff points derived from the mean and standard deviations of Hb values determined from four studies (three of them with less than 25 women) in healthy, iron-sufficient, northern-European pregnant women receiving between 100 and 200 mg of iron as a daily supplement. CDC suggests the cutoff point at the fifth centile.[63] WHO adopted the CDC Hb values and defined anemia as "when individual Hb levels are below two standard deviations of the distribution mean for Hb in an otherwise normal population of the same gender and age who are living at the same altitude."[32] This means that about 5% and 2.5% of a "normal" population would be expected to be classified as anemic depending on the cutoff chosen.

The biological and laboratory variability of Hb level adds to the uncertainty of cutoff points[66] that misclassify more in the "anemic" category than in the "normal" category, because there are usually more subjects just above the cutoff point than just below it. Under field conditions, the standard deviation between Hb determinations must be even higher than in laboratory studies. This misclassification must also be taken into consideration in evaluating the effect of any intervention based on cutoff points. Therefore, the best method for evaluating the effectiveness of interventions is the change in Hb distributions studied by mixed distribution analysis, wherein those with low initial levels essentially move to higher levels, and also by considering the regression to the mean.[24,67]

The suggested CDC/WHO norms for Hb levels in pregnancy[32,63] need revision. A careful analysis of the basis for such norms, based on only four studies with a small number of women of special characteristics, brings out the possibility that the cutoff points for the diagnosis of anemia are too high. A serious attempt to review such norms in a large sample of normal pregnant women must be undertaken. The bases for the derivation of the current norms are rather tenuous for the following reasons:

- Of the four studies mentioned, two from Sweden[55,68] and one each from the UK[69] and Finland,[70] the first three included only 16, 21, and 24 cases, and one that consisted of three different sub-studies, each including 91, 103, and 106 women. The four studies have the virtue of being longitudinal.
- All of the women in these studies received at least 147 daily iron supplements containing from 113 to 200 mg (a total of 16,611 to 29,400 mg!), even though they were well-nourished. There is ample evidence of the development of hemoconcentration with more than 45 daily doses of 60 mg of iron (a total of 2700 mg), as discussed below.
- No analyses of the risk of hemoconcentration were described in the studies selected for derivation of the norms. Based on the mean and standard deviation of the published data, Hb concentration exceeded 130 g/L as early as in the 32nd gestational week and by the 36th week in every study. High Hb carries maternal and perinatal risks, as discussed below.
- The best outcome conditions (birth weight, delivery time, and maternal health) have been reported by other studies to occur when Hb at term is between 95 and 125 g/L.[56-61] Only one of the studies from which the norms were derived gave means and standard deviations of newborn weights. Interestingly, the birth weight in the iron-supplemented group tended to be lower than in the "no therapy" group.[69]

The implications of a norm with a fictitiously iron-induced high Hb can be serious when establishing the diagnosis of anemia and its prevalence in populations and in suggesting control measures for gestational iron deficiency and anemia.

The functional definition of anemia, as its concept implies, is based on impairment of functions due to low Hb concentrations. In the case of pregnancy outcomes, a few detailed studies on the impact of iron deficiency and anemia show that the best outcomes in terms of the characteristics of newborns occur in women who are classified as "anemic" in the third trimester or at term by accepted cutoff levels.[56-61]

The diagnosis of anemia during the second and third trimester of pregnancy is the object of continuous debate because of the normal hemodilution taking place during gestation, and because pregnancy outcomes appear not to be related to Hb levels in the mild to moderate anemia range. A more consistent finding is the association of low Hb values during the first half of pregnancy with low birth weight and preterm delivery, while anemia in the third trimester does not influence the perinatal events unless it is severe.[30,71,72] Scholl et al.[73] clarified that the

association between anemia in general and iron deficiency anemia with preterm delivery was due predominantly to the latter.

An obstacle to many of the studies on gestational iron deficiency is that techniques available for diagnosing iron deficiency are imprecise, given that: a) serum ferritin and transferrin saturation, and even bone-marrow iron, decrease over the course of pregnancy even among women receiving high daily iron doses[55]; b) free erythrocyte protoporphyrins and serum ferritin increase with any inflammatory process such as vaginosis and chorioamnionitis, which are common in pregnancy; and c) reports of levels of serum transferrin receptors vary: some indicate a progressive increase in the course of pregnancy even in the absence of iron deficiency[74] and others report them as stable[75] with increased erythropoietic activity. The proposed method for estimating body iron based on the ratio of transferrin receptors to ferritin[12] that appears to work well in discriminating iron-deficient women from non-iron-deficient women in non-pregnant adult populations and in evaluating the impact of interventions in these population groups may not be discriminating in pregnancy. Results of the TfR/serum ferritin ratio in the non-pregnant woman population from NHANES III reflect two distributions of body iron: one for non-iron-deficient women and one for iron-deficient women. These populations overlap, strengthening the preference of a probabilistic approach based on mixed-distribution analysis over cutoff points.[12,67]

When the TfR/serum ferritin ratio method is applied to pregnant women (as exemplified by an analysis of data from Jamaica[12]), the cumulative frequency distribution represents a single Gaussian-distributed population, with half the women with tissue iron deficiency and a range of body iron stores for women weighing 55 kg of 715 mg (from 275 mg to −440 mg). In the range of body iron deficit (negative iron stores), Hb could be as low as 37 g/L below the mean of 118 g/L for week 29 of pregnancy (118−37 = Hb 81 g/L). Does the single Gaussian distribution indicate that an Hb of 81 g/L is normal in pregnancy? This seems improbable. Does this mean that "iron-deficient" pregnant women do not constitute a different population from "iron-sufficient" pregnant women? Obviously, more research based on functional outcomes is needed.

In summary, the diagnosis of iron deficiency in pregnancy, even by new methods, is not totally reliable. A retrospective view of iron status 4 or more weeks in the postpartum period does not provide data on iron status during the different stages of pregnancy and is affected by blood loss during delivery. The diagnosis of "true" anemia and of iron deficiency in pregnancy thus poses serious problems except when they are severe, which is rare in nutritional iron deficiency. Severe cases are usually complicated by a combination of causes (blood loss, infections, malaria, genetic disorders, etc.).

ARE THERE BENEFITS OF IRON SUPPLEMENTATION DURING PREGNANCY?

Iron supplementation or treatment of mild to moderately anemic pregnant women has proven beneficial in terms of iron status of infants at 3, 6, and 12 months of age, even when supplementation was begun late in pregnancy.[76-78] Maternal and perinatal outcomes due to iron supplementation are less clear. A comprehensive review[79] on the effect of anemia and iron supplementation on pregnancy outcomes indicates that most studies have design flaws. Nonetheless, grouping studies by critical variables (with some leniency on flaws) in order to determine group tendencies on birth weight and preterm delivery in relation to anemia and iron supplementation shows that in developed countries: a) only non-anemic women entered the study; b) most received over 60 mg of iron/d for a variable time during pregnancy; and c) there were no benefits in terms of birth weight or on duration of pregnancy (except in two studies: one in which 30 mg of iron daily was administered to normal women for 8 weeks from gestational week 20[14] and another in which anemic women were supplemented early[80]). Iron nutrition was better in infants born from supplemented women, as indicated above. One study[81] reported higher infant mortality among a group of women receiving universal iron supplementation compared with selectively supplemented anemic women. In contrast, in less-developed countries, most women entering studies were anemic, overall received less iron than women in developed countries, and half (three of six studies in Rasmussen's review[79]) reported some benefit in reducing the incidence of low birth weights. Again, iron nutrition was better in infants born from supplemented women. One study reported that the lengths of newborns of supplemented women were longer, even though their weight was similar to newborns of unsupplemented women.[78]

Rasmussen[79] determined relative and attributable risks of low birth weight and preterm delivery in a few studies; a summary of the results is presented in Table 5. These results show that, in general, moderate gestational anemia carries little risk in developed countries, but that severe anemia carries an overall greater risk, especially for low birth weight over that for preterm delivery, suggesting increased risk of small for gestational age babies. Attributable risk is high for both outcome variables regardless of anemia severity, again being greater for low birth weight than for preterm delivery. The same pattern is observed in pregnant women from less-developed countries. Unfortunately, the number of studies is

Table 5. Relative* and Attributable† Risks for Low Birth Weight and Preterm Delivery by Study Location and Anemia Severity[77]

Location	Relative Risk Low Birth Weight	Relative Risk Preterm Delivery	Attributable Risk Low Birth Weight	Attributable Risk Preterm Delivery
Developed Countries				
Moderate anemia	1.4 (N = 2) (range 0.8–2.1)	1.5 (N = 8) (range 0.6–1.9)	53.0% (N = 1)	45.7% (N = 3) (range 23–67)
Severe anemia	4.6 (N = 3) (range 2.4–6.3)	1.7 (N = 3) (range 1.1–2.6)	71.5% (N = 2) (range 59–84)	32.7% (N = 3) (range 9–59)
Less-Developed Countries				
Moderate anemia	1.5 (N = 8) (range 0.8–3.0)	1.8 (N = 4) (range 0.6–2.6)	6.3% (N = 1)	N/A
Severe anemia	2.6 (N = 9) (range 1.4–5.0)	2.5 (N = 2) (range 1.0–4.0)	52.9% (N = 4) (range 42–83)	N/A

*Relative risk is "the probability of an event occurring in the active group divided by the probability of the event occurring in the control group."

†Attributable risk is "the proportion of the events (in a specified time) in the whole population that may be preventable if a cause of the event were totally eliminated."

very limited for drawing firm conclusions and preterm delivery is reported only if the newborn is of low birth weight, thus reducing its true prevalence. However, from the public health point of view, the importance of preventing anemia is clearly demonstrated.

The general picture emerging from available data suggests the following:

1. Iron deficiency anemia, particularly early in pregnancy, and anemia in general (moderate or severe) have negative effects on pregnancy outcomes that are reduced by iron supplementation.
2. The effect of universal iron supplementation on birth weight and preterm delivery are variable in less-developed countries (different study designs and flaws) and void in women from developed countries who are not anemic early in pregnancy.
3. In populations where iron deficiency is prevalent, the intake of small iron doses plus vitamins early in pregnancy (especially if combined with pre-pregnancy supplement intakes) reduces low birth weight and preterm delivery (the Vietnam study also showed a positive effect[25]).
4. Even among women who appear to be non-anemic and iron sufficient by mid-pregnancy, the intake of small daily doses of iron compared with placebo starting at or before week 20 has a positive effect on both outcome variables, and these positive effects occur even when ferritin levels indicate the absence of iron stores and when Hb levels are suggestive of mild to moderate anemia by current norms.

In summary, adequate iron status and no anemia before the third trimester, preferably as early in pregnancy as possible, are highly desirable.

IS INGESTING MORE IRON THAN WHAT IS NEEDED TO PREVENT IRON DEFICIENCY ANEMIA DESIRABLE AND/OR SAFE?

To our knowledge, there are no studies reporting negative health effects of preconceptional iron-folic acid supplementation, with the exception of gastrointestinal discomfort and the possibility of accidental ingestion of large iron doses, producing iron intoxication, especially in children. However, elevations of plasma "free iron" and of breath ethane excretion rate after daily iron supplement intakes at recommended doses have been reported, indicating the presence of body-reactive iron and oxygen species and elevated lipid peroxidation.[39,40] Lachili et al.[82] also reported increased lipid peroxidation in pregnant women receiving iron and vitamin C supplementation.

A few studies indicate undesirable perinatal and infant developmental outcomes from iron supplementation. A study from Finland among healthy pregnant women found increased infant mortality,[81] and studies from Aberdeen, Scotland[43,56,83] and from other groups,[84] including the CDC,[85,86] suggest that excessive iron supplementation can be dangerous because it increases total Hb concentration above desirable levels and inhibits the normal decline of Hb concentration that reaches a nadir by gestational week 34.

There is renewed interest in determining whether there are negative effects of high Hb concentrations and ferritin levels during pregnancy. In a retrospective study, Murphy et al.[58] showed that both low and high Hb levels increased the rates of premature delivery and low birth weights. In a prospective study, Zhou et al.[61] confirmed that the relationship between Hb levels and risk of poor pregnancy outcome formed a U-shaped curve. In a large,

retrospective analysis of the relationship between Hb, low birth weight, and premature delivery, Steer[59] showed that these undesirable effects increased slowly, with mild to moderate anemia considered to be when Hb was above 115 g/L, and dramatically when Hb levels increased above 130 g/L at any time in pregnancy. These same effects were also observed in other large studies.[60,85] Animal studies also strongly suggest that administering daily iron at the current recommended doses may not be desirable or innocuous.[36,37]

According to recent studies addressing daily and weekly supplementation schemes as proposed by different investigators, the risk of developing hemoconcentration (Hb $>$ 130 g/L) is significantly higher among anemic and non-anemic pregnant women (some iron deficient) ingesting daily iron supplementation at recommended levels than among women ingesting weekly iron.[87] Eckstrom et al.[65] found that 20 doses of 60 mg of iron administered either daily or weekly to anemic Bangladeshi women during gestational weeks 18 to 24 were enough to elevate Hb to desirable levels. An analysis of data from the same study shows that among women receiving 12 weeks of two tablets of 60 mg of iron once weekly, for a total of 24 doses, only two of 72 women (3%) had Hb levels above 130 g/L, while 18 of 66 women (27%) receiving daily doses of 60 mg of iron for 12 weeks had elevated Hb levels. Anemic women appear more prone to overshoot safe Hb levels that carry perinatal risk.

Casanueva and Viteri[87] and Casanueva et al.[88] found that 17.5% of non-anemic Mexican women receiving 60 mg of iron daily for 16 weeks starting at week 20 and 6.7% of non-anemic women receiving two tablets of 60 mg of iron once weekly developed Hb values (adjusted for altitude) greater than 134 g/L. In this study, women developing these high Hb values by weeks 24 to 28 had a significantly higher relative risk of having lower-birth weight infants and preterm deliveries. The negative effect of high Hb in the second trimester was also reported in a large retrospective study.[85] Dr. J.S. Robinson graciously allowed us to analyze data from two unpublished supplementation trials performed in the Maluku province of Indonesia (these same data were used by Beaton and McCabe in their report to the Micronutrient Initiative[89]). This analysis revealed that among 188 pregnant women receiving supervised daily iron (60 mg) and 183 pregnant women receiving weekly iron (two tablets of 60 mg once weekly), there were no women with Hb levels below 90 g/L after the ingestion of 30 tablets containing 60 mg of iron in 24 weeks. The proportion of women developing Hb levels greater than 134 g/L (hemoconcentration) increased exponentially from 6% after 45 tablets to 40% after ingesting 127 of the 60 mg tablets.

The studies in Bangladesh[65] and in Mexico[71] and the data from Maluku suggest that excessive daily iron intake increases the risk of hemoconcentration. In the Mexican study, hemoconcentration was associated with perinatal risk, supporting the suggestion of the Aberdeen[43,56,83] and CDC[85,86] groups. Unfortunately, neither the Bangladesh nor the Maluku studies recorded pregnancy outcomes. A recent Cochrane review of iron and iron folic acid supplementation reinforces these findings.[62] High gestational Hb concentration in response to excessive iron administration appears to be a unique phenomenon during gestation that warrants further in-depth investigations.

Additional studies in Mexican pregnant women receiving 60 mg of iron and 250 μg of folic acid daily for 8 weeks starting either at week 20 or at week 28 showed marked increases in plasma thyobarbituric-acid reacting substances (TBARS). In contrast, women receiving a weekly supplement of 120 mg of iron and 500 μg of folic acid from week 20 to week 28 did not show an increase in TBARS measured 6 days after the previous dose. Moreover, if women with high TBARS at week 28 transferred to the weekly supplementation regimen, TBARS at weeks 32 and 36 fell to levels similar to those prior to iron supplementation (gestational week 20).[89]

Similar to the debate on how to diagnose iron deficiency and anemia in pregnancy is the debate on the benefits of routine daily iron supplementation during pregnancy at levels currently recommended by different agencies.[31,32,63] It appears that small daily doses as recommended by the US Food and Nutrition Board[90] and the US Institute of Medicine,[91] as well as weekly dosing starting early in pregnancy, are safer and essentially as efficacious as daily iron in preventing iron deficiency and improving iron nutrition when adherence is satisfactory.

CONCLUSION

Field research to further validate the "preventive supplementation" approach with intermittent iron-folic acid supplementation is needed. The studies should address women's needs during pregnancy and during their lifetimes as long as they are a group particularly at risk of iron deficiency and anemia. Long-term weekly supplementation with iron and folic acid to non-pregnant women can also bring benefits in terms of the prevention of neural tube defects and hyperhomocysteinemia.[92,93] Further supplementation with vitamin A and possibly with multiple minerals and vitamins may bring further health benefits.[94,95] Weekly dosing is more economical and more manageable in community settings given its greater safety, and can be managed under the supervision of health establishments and given to community organizations, schools, religious groups, markets, factories, etc., thus increasing coverage of vulnerable groups.[41,96]

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REFERENCES

1. United Nations Administrative Committee on Coordination Sub-Committee on Nutrition, International Food Policy Research Institute. *4th Report on the World Nutrition Situation. Nutrition Throughout the Life Cycle*. January 2000; Geneva, Switzerland. Available online at: http://www.unsystem.org/scn/Publications/4RWNS/4rwns.pdf. Accessed October 27, 2005.
2. Mason JB, Lotfi M, Dalmiya N, et al. *The Micronutrient Report: Current Progress in the Control of Vitamin A, Iodine, and Iron Deficiencies. Ottawa, Ontario, Canada: The Micronutrient Initiative International Development Research Center; 2001.* Available online at: http://www.micronutrient.org/resources/publications/The%20Micronutrient%20Report.htm. Accessed October 27, 2005.
3. Kim I, Hungerford DW, Yip R, et al. Pregnancy nutrition surveillance system—United States, 1979-1990. MMWR CDC Surveill Summ. 1992;41:25–41.
4. Bodnar LM, Scanlon KS, Freedman DS, et al.. High prevalence of postpartum anemia among low-income women in the United States. Am J Obstet Gynecol. 2001;185:438–443.
5. Viteri FE, Ali F, Tujague J. Long-term weekly iron supplementation improves and sustains non-pregnant women's iron status as well or better than currently recommended short-term daily supplementation. J Nutr. 1999;129:2013–2020.
6. Yip R. Iron deficiency: contemporary scientific issues and international programmatic approaches. J Nutr. 1994;124:1479S–1490S.
7. Morasso M del C, Molero J, Vinocur P, et al. Iron deficiency and anemia in pregnant women from Chaco, Argentina. Arch Latinoam Nutr. 2002;52:336–343.
8. Bothwell TH, Charlton R. *Iron Deficiency in Women*. Washington, DC: International Nutritional Anemia Group; 1981.
9. Hallberg L. Iron balance in pregnancy and lactation. In: Fomon SJ, Zlotkin S, eds. *Nutritional Anemias*. New York: Vevey/Raven Press; 1992.
10. Fernandez-Ballart J. Iron metabolism during pregnancy. Clin Drug Invest. 2000;19(suppl 1):9–19.
11. Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency of the US population. Blood. 1986;68:726–731.
12. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003;101:3359–3364.
13. Kaufer M, Casaneuva E. Relation of pregnancy serum ferritin levels to Hb levels throughout pregnancy. Eur J Clin Nutr. 1990;44:709–715.
14. Ronnenberg AG, Wood RJ, Wang X, et al. Preconception Hb and ferritin concentrations are associated with pregnancy outcome in a prospective cohort of Chinese women. J Nutr. 2004;134:2586–2591.
15. Franzetti S, Mejía LA, Viteri FE, Alvarez A. Body iron reserves of rural and urban Guatemalan women of reproductive age. Arch Latinoamer Nutr. 1984;34:69–82.
16. Viteri FE, Alvarez E, Batres R, et al. Fortification of sugar with NaFeEDTA improves iron status in semirural populations in Guatemala. Am J Clin Nutr. 1995;61:1153–1163.
17. Thuy PV, Berger J, Davidsson L, et al. Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. Am J Clin Nutr. 2003;78:284–290.
18. Thuy PV, Berger J, Nakanishi Y, et al. Effectiveness of NaFeEDTA fortified fish sauce in controlling iron deficiency in Vietnam. Abstract presented at the International Nutritional Anemia Group meeting; Lima, Peru; November 18, 2004.
19. Viteri FE. Iron supplementation for the control of iron deficiency in populations at risk. Nutr Rev. 1997;55:195–209.
20. Zlotkin SH, Christofides AL, Hyder SM, et al. Controlling iron deficiency anemia through the use of home-fortified complementary foods. Ind J Pediatr. 2004,71:1015–1019.
21. Smuts CM, Lombard CJ, Benadé S, et al. International Research on Infant Supplementation (IRIS) Study Group. Efficacy of a foodlet-based multiple micronutrient supplement for preventing growth faltering, anemia, and micronutrient deficiency of infants: The Four Country IRIS Trial Pooled Data Analysis J Nutr. 2005;135:631–638.
22. Hop LT, Berger J. Multiple micronutrient supplementation improves anemia, micronutrient status and growth of Vietnamese infants: double blind, randomized, placebo-controlled trial. J Nutr. 2005;135:660S–665S.
23. Angeles-Agdeppa I, Schultink W, Sastroamidjojo S, et al. Weekly micronutrient supplementation to build iron stores in female Indonesian adolescents. Am J Clin Nutr. 1997;66:177–183.
24. Tee ES, Kandiah M, Awin N, et al. School-administered weekly iron-folate supplements improve Hb and ferritin concentrations in Malaysian adolescent girls. Am J Clin Nutr. 1999;69:1249–1256.
25. Berger J, Thanh HTK, Cavalli-Sforza T, et al. Community mobilization and social marketing to promote weekly iron-folic acid supplementation in women of reproductive age in Vietnam: impact on anemia and iron status. Nutr Rev. 2005;63(12 part 2):S95–S108.
26. Angeles-Agdeppa I, Paulino LS, Ramos AC, et al. Government-industry partnership in weekly iron-folic acid supplementation for women of reproductive age: impact on iron status. Nutr Rev. 2005;63(12 part 2):S109–S115.
27. Crape BL, Kenefick E, Cavalli-Sforza T, Busch-Hallen J, Milani S, Kanal K. Positive impact of a weekly iron-folic acid supplement delivered with social marketing to Cambodian women: compliance, participation, and hemoglobin levels increase with higher socioeconomic status. Nutr Rev. 2005;63(12 part 2):S134–S138.
28. DeMaeyer EM, ed. *Preventing and Controlling Iron*

Deficiency Anaemia through Primary Health Care. Geneva: World Health Organization; 1989; 58 pp.
29. Viteri FE. Prevention of iron deficiency. In: Howson CP, Kennedy E, Horwitz A, eds. *Micronutrient Deficiencies: A Toolkit for Policymakers and Public Health Workers*. Washington, DC: Institute of Medicine, National Academies Press; 1998; 45–102.
30. Scholl TO, Hediger ML, Bendich A, et al. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. Am J Epidemiol. 1997;146:134–141.
31. Stoltzfus RJ, Dreyfus ML, eds. *INACG-WHO-UNICEF Guidelines for the Use of Supplements to Prevent and Treat Iron Deficiency Anemia*. Washington, DC: ILSI Press; 1998; 39 pp.
32. WHO/UNICEF/UNU. *Iron Deficiency Anaemia. Assessment, Prevention, and Control. A guide for programme managers*. Geneva: World Health Organization; 2001; 114 pp.
33. Goodnough LT, Skikne B, Brugnara C. Erythropoietin, iron, and erythropoiesis. Blood. 2000;96:823–833.
34. Viteri FE, Liu XN, Martin A, Tolomei K. True absorption and retention of supplemental iron is more efficient when administered every-three-days rather than daily to iron-normal and iron-deficient rats. J Nutr. 1995;125:82–91.
35. Viteri FE. Suplementacion con hierro para el control de la deficiencia de hierro en poblaciones en riesgo. In: O'Donnell AM, Viteri FE, Carmuega E, eds. *Deficiencia De Hierro: Desnutricion Oculta en America Latina*. Buenos Aires, Argentina: Centro de Estudios sobre Nutricion Infantil; 1997; 231–257.
36. Knutson MD, Walter PB, Ames BN, Viteri FE. Both iron deficiency and daily iron supplements increase lipid peroxidation in rats. J Nutr. 2000;130:621–628.
37. Walter PB, Knutson MD, Paler-Martinez A, et al. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. Proc Natl Acad Sci U S A. 2002;99:2264–2269.
38. Srigiridhar K, Nair KM, Subramanian R, Singotamu L. Oral repletion of iron induces free radical mediated alterations in the gastrointestinal tract of rat. Mol Cell Biochem. 2001;219:91–98.
39. Breuer W, Ronson A, Slotki IN, et al. The assessment of serum nontransferrin-bound iron in chelation therapy and iron supplementation. Blood. 2000; 95:2975–2982.
40. Mertz SD, Woodhouse LR, Donangelo CM, et al. Breath ethane excretion rate in young women is increased in daily iron but not in daily zinc supplementation. FASEB J. 1999;13:A216.
41. Viteri FE. Iron deficiency in children: new possibilities for its control. Int Child Health. 1995;6:49–62.
42. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr. 2000; 72(suppl 1):257S–264S.
43. Hytten FE, Leitch I, Baird D. *The Physiology of Human Pregnancy*. 2nd ed. Oxford: Blackwell; 1971; 1–43.
44. Viteri FE, García Ibáñez R, Torún B. Sodium iron NaFeEDTA as an iron fortification compound in Central America: absorption studies. Am J Clin Nutr. 1978;31:961–971.
45. O'Brien KO, Zavaleta N, Caulfield LE, et al. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. Am J Clin Nutr. 1999;69:509–515.
46. Barrett JF, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. BMJ. 1994;309:79–82.
47. Letsky EA. Erythropoiesis in pregnancy. J Perinat Med. 1995;23:39–45.
48. Hytten F. Blood volume changes in normal pregnancy. Clin Haematol. 1985;14:601–612.
49. Viteri FE. Iron supplementation as a strategy for the control of iron deficiency and ferropenic anemia. Arch Latinoam Nutr. 1999;49(3 suppl 2):15S–22S.
50. Cook JD, Reddy MB. Efficacy of weekly compared with daily iron supplementation. Am J Clin Nutr. 1995;62:117–120.
51. Pizarro F, Olivares M, Arredondo M, et al. *Does Daily Iron Administration Produce a Mucosal Blockade?* Wageningen, The Netherlands: European Academy of Nutritional Sciences; 1997.
52. Hallberg L. Combating iron deficiency: daily administration of iron is far superior to weekly administration. Am J Clin Nutr. 1998;68:213–217.
53. Beard JL. Weekly iron intervention: the case for intermittent iron supplementation. Am J Clin Nutr. 1998;68:209–212.
54. Viteri FE, Mendoza C, Guiro A, et al. Daily and weekly supplementation and reference-dose iron (Fe) absorption in Berkeley, Ca. and Dakar, Senegal. FASEB J. 1999;13:A536.
55. Svanberg B. Absorption of iron in pregnancy. Acta Obstet Gynecol Scand Suppl. 1975;48:1–108.
56. Mahomed K, Hytten F. Iron and folate supplementation in pregnancy. In: Chalmers I, ed. *Effective Care in Pregnancy*. Oxford: Oxford University Press; 1989; 301–307.
57. Steer P, Alam MA, Wadsworth J Welch A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ. 1995; 310:489–491.
58. Murphy JF, O'Riordan J, Newcombe, et al. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet. 1986;1(8488):992–995.
59. Steer PJ. Maternal hemoglobin concentration and birth weight. Am J Clin Nutr. 2000;71(suppl 5): 1285S–1287S.
60. Xiong X, Buekens P, Alexander S, et al. Anemia during pregnancy and birth outcome: a meta-analysis. Am J Perinatol. 2000;17:137–146.
61. Zhou LM, Yang WW, et al. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am J Epidemiol. 1998;148:998–1006.
62. Peña-Rosas JP, Viteri FE. Routine oral iron supplementation with or without folic acid for women during pregnancy. An update. Cochrane Database Syst Rev. 2005; in press.
63. CDC criteria for anemia in children and childbearing-age women. MMWR Morb Mortal Wkly Rep. 1989;38:400–404.
64. Beaton GH. Iron needs during pregnancy: do we need to rethink our targets? Am J Clin Nutr. 2000; 72:265S–271S.
65. Ekstrom EC, Hyder SM, Chowdhury, et al. Efficacy

and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues. Am J Clin Nutr. 2002;76:1392–1400.
66. Borel MJ, Smith SM, Derr J, Beard JL. Day-to-day variation in iron-status indices in healthy men and women. Am J Clin Nutr. 1991;54:729–735.
67. Cohen JH, Haas JD. The comparison of mixed distribution analysis with a three-criteria model as a method for estimating the prevalence of iron deficiency anaemia in Costa Rican children aged 12-23 months. Int J Epidemiol. 1999;28:82–89.
68. Sjostedt, JE, Manner P, Nummi S, Eknved G. Oral iron prophylaxis during pregnancy: a comparative study on different dosage regimens. Acta Obstet Gynecol Scand Suppl. 1980;95:43–51.
69. Taylor DJ, Mallen C, McDougall N, Lind T. Effect of iron supplementation on serum ferritin levels during and after pregnancy. Br J Obstet Gynecol. 1982;89: 1011–1017.
70. Puolakka J, Janne O, Pakarinen A, et al. Serum ferritin as a measure of iron stores during and after normal pregnancy with and without iron supplements. Acta Obstet Gynecol Scand Suppl. 1980;95:43–51.
71. Rush D. Nutrition and maternal mortality in the developing world. Am J Clin Nutr. 2000;72(suppl): 212S–240S.
72. Klebanoff MA, Shiono PH, Selby JV, et al. Anemia and spontaneous preterm birth. Am J Obstet Gynecol. 1991;164:59–63.
73. Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr. 1992;55:985–988.
74. Choi JW, Im MW, Pai SH. Serum transferrin receptor concentrations during normal pregnancy. Clin Chem. 2000;46:725–727.
75. Milman N, Bergholt T, Eriksen L, et al. Iron prophylaxis during pregnancy: how much iron is needed? A randomized dose-response study of 20-80 mg ferrous iron daily in pregnant women. Acta Obstet Gynecol Scand. 2005;84:238–247.
76. Puolakka J, Janne O, Vihko R. Evaluation by serum ferritin assay of the influence of maternal iron stores on the iron status of newborns and infants. Acta Obstet Gynecol Scand Suppl. 1980;95:53–60.
77. Colomer J, Colomer C, Gutierrez D. Anemia during pregnancy as a risk factor for infant iron deficiency. Report from the Valencia infant anemia cohort. In: Hercberg S, Galan P, Dupin H, eds. *Recent Knowledge on Iron and Folate Deficiencies in the World*. Colloque INSERM; 1990; 577–582.
78. Preziosi P, Prual A, Galan P, et al. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr. 1997;66:1178–1182.
79. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr. 2001;131(suppl 2):590S–603S.
80. Menendez C, Todd J, Alonso PL, et al. The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. Trans R Soc Trop Med Hyg. 1994;88:590–593.
81. Hemminki . Routine administration of iron and vitamins during pregnancy: a review of controlled clinical trials. Br J Obstet Gynaecol. 1978;85:404–410.
82. Lachili B, Hininger I, Faure H, et al. Increased lipid peroxidation in pregnant women after iron and vitamin C supplementation. Biol Trace Elem Res. 2001; 83:103–110.
83. Lund CJ, Donovan JC. Blood volume during pregnancy. Am J Obstet Gynecol. 1967;98:393–397.
84. Wallenburg HCS, van Eijk HG. Effect of oral iron supplementation during pregnancy on maternal and fetal iron status. J Perinat Med. 1984;12:7–12.
85. Scanlon KS, Yip R, Schieve LA, Cogswell ME. High and low hemoglobin levels during pregnancy: differential risks for preterm birth and small for gestational age. Obstet Gynecol. 2000;96(5 part 1):741–748.
86. Huisman A, Aarnoudse JG. Increased 2nd trimester hemoglobin concentration in pregnancies later complicated by hypertension and growth retardation. Acta Obstet Gynecol Scand. 1986;65:605–608.
87. Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. J Nutr. 2003;133(5 suppl 2):1700S–1708S.
88. Casanueva E, Erazo B, Rosales X, et al. Anemia, oxidative stress and iron supplementation on pregnant women. FASEB J. 2005;A3174.
89. Beaton G, McCabe GP. *Efficacy of Intermittent Iron Supplementation in the Control of Iron Deficiency Anemia in Developing Countries: An Analysis of Experience*. Ottawa, Ontario, Canada: Micronutrient Initiative; 2001.
90. Anderson SA, ed. *Guidelines for the Assessment and Management of Iron Deficiency in Women of Childbearing Age*. Bethesda, MD: Federation of American Societies for Experimental Biology; 1991.
91. Institute of Medicine. *Iron-Deficiency Anemia: Recommended Guidelines for the Prevention, Detection, and Management Among U.S. Children and Women of Childbearing Age*. Washington, DC. National Academies Press; 1993.
92. Martinez de Villarreal L, Perez JZ, Vazquez PA, et al. Decline of neural tube defects cases after a folic acid campaign in Nuevo Leon, Mexico. Teratology. 2002;66:249–256.
93. Adank C, Green TJ, Skeaff CM, Briars B. Weekly high-dose folic acid supplementation is effective in lowering serum homocysteine concentrations in women. Ann Nutr Metab. 2003;47:55–59.
94. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr. 2001;13:85–90.
95. Thu BD, Schultink W, Dillon D, et al. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. Am J Clin Nutr. 1999;69:80–86.
96. Viteri FE. A new concept in the control of iron deficiency (ID): community-based preventive supplementation (PS) of at-risk groups by weekly intake of iron supplements. Biomed Environ Sci; 1998;11:46–60.