



## TRUE ABSORPTION OF SUPPLEMENTAL IRON

83

weather-Tait et al. 1985, Hahn et al. 1943, Swart et al. 1950); and 2) the fact that iron absorption is directly related to the degree of iron deficiency (Bothwell et al. 1979). Absorption of therapeutic iron in three successive 10-d periods in iron-deficient humans has been shown to decrease as iron depletion occurs (Norrby and Solvell 1974). More recently, evidence has been provided that intermittent dosing is as effective in correcting iron nutrition in iron-deficient, anemic rats (Viteri et al. 1992, Light and Southon 1990).

The present studies were designed to study in detail the sequential changes in true iron absorption and total iron retention in iron-deficient and iron-normal rats ingesting daily doses (simulating the recommended scheme for iron supplementation during pregnancy in the developing world) and in rats administered the same doses every third day. The reasoning behind the every third day schedule was that, in rats, small intestinal cell renewal occurs every 2-3 d (Holt et al. 1983) and that the blocking effect of high dietary intake lasted 3 d (Fairweather-Tait et al. 1985), so that by administering supplementary iron at a similar timing as gastrointestinal cell renewal the blocking effect of previous iron boluses would be avoided, and true absorption and retention would be more efficient. The intermittent schedule would also reduce gastrointestinal iron overload due to unassimilated iron, which may be related to the undesirable side effects reported in humans supplemented daily (Charoenlarp et al. 1988, Gillespie et al. 1991, Hallberg et al. 1966, Kuizon et al. 1983, World Health Organization 1990).

## MATERIALS AND METHODS

A total of 92 weanling (21-26 d old) male Sprague-Dawley rats [mean overall weight 46 g  $\pm$  (SD) 4.6 g; Martin and Kingman, Fremont, CA] were individually housed in wire-bottomed stainless steel cages, with a 12-h light cycle and 22°C temperature-controlled environment. Sixty-four rats were used in the first study and 28 in the second. Rats were given free access to deionized water. These conditions remained unchanged throughout the studies. Animal housing conditions, continued care and experimental procedures were approved by the University of California at Berkeley's Animal Use Committee.

Upon arrival, all the rats were given free access to Purina laboratory rodent diet (#5001, Purina, St. Louis, MO) for 1 d. Rats were then assigned to four groups (first study) or six groups (second study), with the groups matched by body weight in each study (Fig. 1). Half of these groups received an iron-deficient diet (the AIN-76 diet without ferric citrate as iron source, Fe concentration 9.3  $\pm$  0.9  $\mu$ g/g); the other groups received the complete AIN-76 diet for 3 d.

From then on, the rats were trained to meal-feed for 1.5 h twice daily (0730 and 1730 h) and were fed the iron-deficient diet preceded by a 0.8-g premeal of sucrose and iron-deficient diet (50:50). The premeal, served on a small glass dish, contained either no added iron [iron-deficient (D)<sup>5</sup> groups] or 400  $\mu$ g or elemental iron as reagent-grade FeSO<sub>4</sub>·7H<sub>2</sub>O twice daily [iron-normal (N) groups]. The rats were given 15 min to consume the premeal, and those that had consumed it fully were then given free access to the iron-deficient diet. Those that had some premeal left on the dish did not receive the iron-deficient diet, and at the following feeding time were again offered a premeal and the process repeated. In 2-3 d all rats learned to eat all their premeal. These iron-deficient or iron-normal regimens were continued for a total of 12 d.

In the first study, half the rats in the D groups and half the rats in the N groups continued on the same regimens for the next 20-22 d of study (the DD and NN groups, respectively). The other half of each D and N group began receiving the premix supplemented twice daily with five times the normal iron content, to provide, in two daily doses, the equivalent of 10 times the normal daily iron intake. These groups were designated the iron-deficient, daily supplemented group (D1S) and the iron-normal, daily supplemented group (N1S), respectively. The desired total iron intakes from the two daily premeals for the respective D, N and supplemented (D1S and N1S) groups were <20, 800 and 8000  $\mu$ g of elemental iron per day. The supplemental iron level (10 times the usual intake) would be similar to the recommended daily intakes of 120 to 180 mg elemental iron/d by pregnant women.

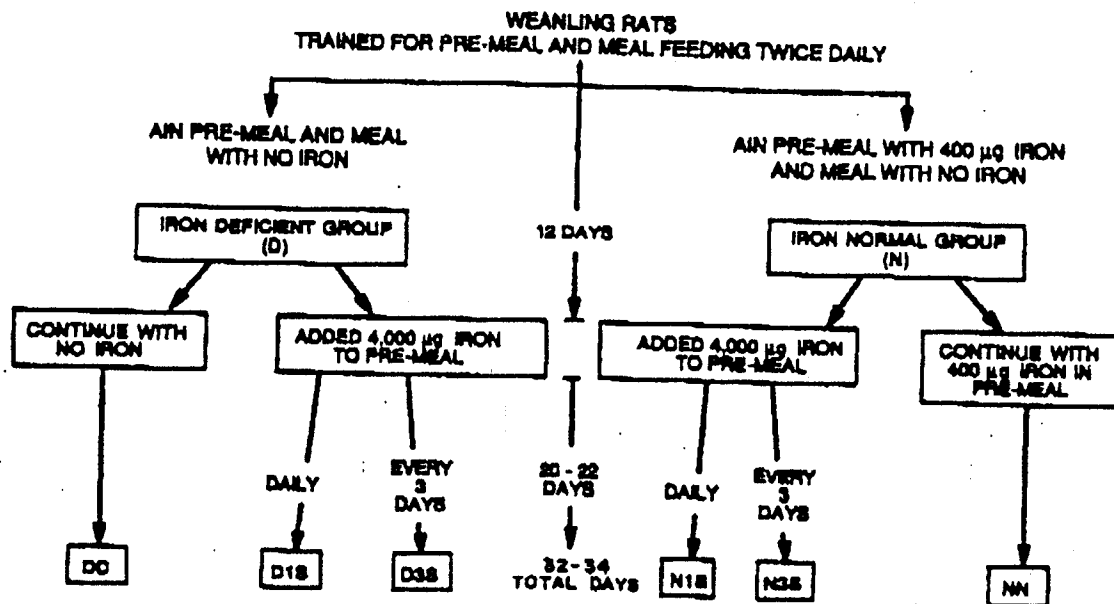
In the second study, groups DD, NN, D1S and N1S were treated the same as the corresponding groups in the first study, and two additional groups received the same supplemented premeal as in the first study, but only every 3 d. The 2 d in between, these groups of rats received either iron-deficient (group D3S) or iron-normal premeals (group N3S). Thus, the total supplemental iron intake of the D3S and N3S groups was 33% of that in groups D1S and N1S, but total iron intake in group N3S was 45% that of group N1S because of the "normal" iron intake in the days between supplemental iron doses.

All the animals were inspected daily and weighed twice weekly. Food intake was estimated weekly by

<sup>5</sup>Group abbreviations: DD, rats kept iron-deficient throughout the study (unsupplemented); NN, rats kept iron-normal throughout the study (unsupplemented); D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study.

VITERI ET AL.

## EXPERIMENTAL DESIGN



**FIGURE 1** Experimental design. Rats were 22 to 27 d of age when iron-deficient diets were started. Iron supplementation was started on d 13 of the study. Group abbreviations: Group DD, rats kept iron-deficient throughout the study (un-supplemented); Group D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; Group D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study (un-supplemented); Group N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; Group N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; Group NN, rats kept iron-normal throughout the study (un-supplemented).

the difference in weight of individual food containers minus spilled food on two consecutive days. Growth was correlated significantly with food intake ( $r = 0.886$ ). Total iron intake from the iron-deficient diet in these containers ranged from 74 to 102  $\mu\text{g}/\text{d}$  for the DD group and from 93 to 140  $\mu\text{g}/\text{d}$  for all other groups. The lowest intakes occurred in the first week of study and the highest in the last week of study in all groups.

In the first study, iron deficiency and its recovery was evaluated by hemoglobin determinations from blood obtained by tail snipping on d 0, 2, 8 and 15 after iron supplementation started, and again on d 20-22, when the rats were killed by pentobarbital injection and exsanguination from aortic blood collection. In the second study, hemoglobin was measured only at the end of the study. In Study 1, two rats in the NN and DD groups and eight rats in the N1S and D1S groups were killed earlier to study intestinal and liver iron contents at intermediate points of the study (to be reported in another paper).

True iron absorption and daily rates of loss of the different iron doses were measured by mixing thoroughly 15.12 kBq of  $^{59}\text{Fe}$  contained in  $<1 \mu\text{g}$  of elemental iron as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (Amersham International, Amersham, Buckinghamshire, U.K.) to the iron in the premeal. One hour after the rats had consumed the premeal, followed by the meal, whole-body radioactivity was measured in duplicate for 2 min in an animal whole-body counter [IAEC 12-cm

two synchronous NaI crystal, lead-shielded, 10-stage venetian blind-type Ortec detector (A5788); Lawrence Berkeley Laboratories, Berkeley, CA], previously standardized for rat body size, geometry and  $^{59}\text{Fe}$  peak counting. The 1-h radioactivity measurement was established as 100% of ingested  $^{59}\text{Fe}$ .

Mean overall residual radioactivity in the premeal dishes was 4.30% (SD 3.79%). After the first measurement, all rats were measured for 2 min twice daily for seven consecutive days in the first study to collect information on the components of biological decay (corrected for physical decay). Counting times were lengthened, when needed, to obtain  $<1\%$  CV between duplicate counts.

Logarithmically transformed counts allowed the straight line regression of the points measured in 4 to 7 d to the time 1 h of the day of  $^{59}\text{Fe}$  administration, in order to calculate the percentage of true iron absorption of the corresponding premeal (antilog of 1 h intercept). The rapid fall of radioactivity for the first 3 d represented the excretion of radioactive iron contained in the intestinal lumen and in sloughed mucosal cells. Therefore, counts were obtained 1 h after radioactive iron ingestion and again on 4 to 7 d in the second study.

Radioactive iron rates of loss were estimated by calculating the biological decay constant in the segment with a straight regression line from the

## TRUE ABSORPTION OF SUPPLEMENTAL IRON

85

TABLE 1

*Days of experiment and supplement doses when iron absorption was measured in rats<sup>1</sup>*

NN and DD experiment	Groups N1S and D1S			Groups N3S and D3S		
	Day of experiment	Day of supplementation	Supplement dose	Day of experiment	Day of supplementation	Supplement dose
	13 <sup>2</sup>	1	1 <sup>3</sup>	13	1	1
	14	2	3			
	15	3	5			
	16	4	7	16	4	3
	19	7	13	19	7	5
	20	8	15			
	22	10	19	22	10	7
	25	13	25	25	13	9

Group abbreviations: NN, rats kept iron-normal throughout the study (unsupplemented), DD, rats kept iron-deficient throughout the study (unsupplemented), N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study, D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study. Supplementation was begun on d 13 of experiment.

<sup>1</sup>Doses of supplement were administered twice every day (N1S and D1S) or twice every 3 d (N3S and D3S) in the premeals.

Exponential transformation of the daily counts obtained from d 4 to 7. This decay constant [100(1 - e<sup>-k</sup>)/k] is expressed as the percent decay of the total radioactive iron pool per day.

The true iron absorbed was measured in the study days indicated in Table 1. This table also presents the days of supplementation and the supplement dose that had <sup>59</sup>Fe.

Most animals in both experiments had iron absorption measured on more than one occasion separated by at least 7 d, after rates of iron loss were stable. <sup>59</sup>Fe absorption and iron rates of loss on subsequent occasions were corrected for residual radioactivity from previous radioactive iron administrations.

The data was analyzed by one-way ANOVA to detect an experimental treatment effect at the same evaluation times ( $P < 0.05$ ), and, if this was significant, differences between individual treatments were identified by Scheffé contrasts (Scheffé 1959). In the cases in which iron absorption and rates of iron loss were studied more than once in the same animals, the within-animal effect was evaluated at the same points within treatments, by analysis of covariance. There were no within-animal effects. Analyses were performed using SPSS PC Plus base system (SPSS Inc., Chicago, IL).

## RESULTS

**Clinical and food intake evaluations.** All the animals remained healthy throughout the study, even though the group D rats in both studies began to appear pale and exhibited a coarse and somewhat

sparse coat at d 10-12. These characteristics disappeared in the first few days of supplementation in groups D1S and D3S, whereas they became more evident in groups DD as the study progressed.

In general, rats consumed their premeals completely. However, the groups receiving daily supplementation (groups D1S and N1S) usually took longer to consume the supplemented premeal, and 7-9% of the time they did not completely consume the premeal. Food intake in the iron-normal groups, supplemented or not, was as expected based on the rats' weight at different periods of study. The D groups' intake was 18% lower than that of the N groups ( $P < 0.01$ ) by the second week of deficient iron intake. This decline in intake continued so that by the end of the study the DD group's intake was 68% that of the NN group ( $P < 0.01$ ). Food intake in D1S and D3S rats began to increase when supplementation started, reaching 88% of the intakes of rats in the N groups by the end of the study ( $P > 0.05$ ).

**Growth.** Table 2 shows the weight of rats by groups and by phases of the study, before and after supplementation was begun. All iron-normal supplemented rats (N1S and N3S groups) are reported together because their weights did not differ in the different stages of these studies. The same applies to all iron-deficient supplemented rats (groups D1S and D3S). The first phase (prior to supplementation) lasted 12 d, and the second phase between 20 and 22 d. It is evident that iron-deficient rats grew significantly less than iron-normal rats, this weight deficit increased in groups DD as the study progressed. The deficient rats that became supplemented (Groups D1S and D3S) showed "catch-up growth," approaching the iron-normal groups by the end of the study, independent of

TABLE 2  
Body weight of rats by experimental groups and days of experiment<sup>1</sup>

Groups <sup>2</sup>	Prior to supplementation		During supplementation	
	Day 1 of experiment	Day 12 of experiment	Day 22-24 of expt., d 10-12 of supplement	Day 32-34 of expt., d 20-22 of supplement
NN	49.0 ± 1.4 (16)	103.9 ± 2.4 <sup>a</sup> (16)	129.4 ± 3.2 <sup>a</sup> (14)	146.0 ± 3.8 <sup>a</sup> (14)
DD	48.6 ± 1.7 (16)	81.0 ± 1.4 <sup>c</sup> (30)	95.7 ± 2.2 <sup>c</sup> (14)	105.8 ± 2.2 <sup>b</sup> (14)
N1S-N3S	48.2 ± 1.6 (30)	96.8 ± 1.2 <sup>ab</sup> (30)	124.1 ± 2.2 <sup>a</sup> (22)	141.6 ± 2.7 <sup>a</sup> (22)
D1S-D3S	48.5 ± 0.9 (30)	86.9 ± 1.3 <sup>b</sup> (30)	110.0 ± 2.5 <sup>b</sup> (22)	130.5 ± 2.8 <sup>a</sup> (22)

<sup>1</sup>Values are means ± sz with the number of rats in parentheses. Within a column, different superscripts indicate significant differences at the same evaluation time ( $P < 0.05$ ).

<sup>2</sup>Group abbreviations: NN, rats kept iron-normal throughout the study (unsupplemented); DD, rats kept iron-deficient throughout the study (unsupplemented); N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study.

the frequency of supplement intake. All the iron-normal groups (NN, N1S and N3S) grew similarly, independent of supplementation and its modality.

**Hemoglobin concentrations.** In a pilot study, hemoglobin concentrations were measured every 2 d from the start in weanling rats fed an iron-deficient diet and compared with that in normal animals. It was evident that hemoglobin concentrations were significantly lower by d 10 of iron-deficient intake. Therefore supplementation was started on d 13. Hemoglobin concentrations from this day on in the different groups are shown in Table 3. Study 1 demonstrated that hemoglobin concentrations prior to supplementation in the deficient rats were significantly lower than in the iron-normal rats and that they declined further in the DD group as iron deficiency became more severe during the supplementation phase of the study. In contrast, the hemoglobin concentration began to increase in the D1S group already by d 2 of supplementation, reaching levels similar to those in the N groups already by d 8 (data not shown). From then on hemoglobin concentrations were similar among N and D1S groups. Hemoglobin concentrations in the second study were measured only at the end of the study and resembled those in the first study.

**True iron absorption.** Table 4 presents the sequential results of true iron absorption of the premeal iron, expressed as the percentage of dose administered.

Both unsupplemented groups (NN and DD) absorbed the iron in their corresponding premeals at a constant rate throughout the study (means, 34.2% and 89.7%, respectively).

Iron-normal rats supplemented daily and every 3 d (groups N1S and N3S) absorbed the first supplemental dose at a 10.1% level. The absorption of supplemental

TABLE 3  
Hemoglobin concentration by experimental groups of rats and days of experiment<sup>1</sup>

Time point	Group <sup>2</sup>	n	Hemoglobin g/L
Day 12 <sup>3</sup>	N	22	145.5 ± 2.1 <sup>a</sup>
	D	22	112.0 ± 3.7 <sup>b</sup>
Day 32-34	NN	12	155.8 ± 3.2 <sup>a</sup>
	DD	12	70.9 ± 1.8 <sup>b</sup>
	N1S	10	155.0 ± 3.0 <sup>a</sup>
	D1S	10	161.9 ± 1.1 <sup>a</sup>
Day 32-34	NN	2	154.5, 151.1 <sup>a</sup>
	DD	2	71.0, 67.4 <sup>b</sup>
	N1S	6	159.7 ± 2.7 <sup>a</sup>
	N3S <sup>4</sup>	5	154.0 ± 1.4 <sup>a</sup>
	D1S	6	154.7 ± 4.3 <sup>a</sup>
	D3S	6	160.0 ± 2.4 <sup>a</sup>

<sup>1</sup>Values are means ± SE; individual values are given when  $n = 2$ . Within a study and at the same time point, values with different superscripts are different ( $P < 0.05$ ).

<sup>2</sup>Group abbreviations: NN, rats kept iron-normal throughout the study (unsupplemented); DD, rats kept iron-deficient throughout the study (unsupplemented); N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study.

<sup>3</sup>The d 12 values include all the iron-normal (N) and iron-deficient (D) rats before they were separated into NN and N1S, and DD and D1S.

<sup>4</sup>One rat was excluded because of a technical error in obtaining the blood sample.

## TRUE ABSORPTION OF SUPPLEMENTAL IRON

87

in the N1S group fell to reach a stable level between 5 and 7% from around d 7 on. Logarithmic transformation of percent absorbed iron against time (days) resulted in a straight line with an intercept of 9.74% absorption. In contrast, iron-normal rats supplemented every 3 d (group N3S) absorbed the supplemental iron at a very constant rate throughout the period of supplementation (overall mean, 9.5%). Iron-deficient rats supplemented daily or every 3 d (groups D1S and D3S) absorbed the first dose of supplemental iron at a mean level of 22.6%. This relatively high absorption level fell very rapidly in the D1S group to reach a mean level of only 12.8% by d 2 (dose 3), 11.8% by d 3 (dose 5), between 7 and 8% by d 4 (dose 7) and 10 of supplementation (doses 15-19) and ~5% by d 13 (dose 25). Logarithmic transformation of the percentage of absorbed iron against time also resulted in a negative straight line with an intercept at 15.60% absorption.

Iron-deficient rats supplemented every 3 d (group D3S) showed a more sustained level of absorption, being still 19.4% by d 4 (dose 3), and reaching, in a slow and almost straight negative linear fashion, 2% absorption by d 13 (dose 9). The intercept, calculated by logarithmic transformation of the data, was 25.33% absorption.

Figure 2 presents in graphic form the iron absorption results of the different supplemented groups. This figure clearly shows that results of the two studies form a continuum in the N1S and D1S groups, which show a rapid decline.

**Radioactive iron rates of loss.** Table 5 presents the data obtained from each group of treated rats. The rates of iron loss tended to decrease slightly with time in the supplemented groups, without reaching significant differences ( $P = 0.15$ ). The data for groups NN, DD, N1S and D1S represent combined data from the two studies because they were statistically similar in both studies.

Beginning from a small rate of loss in the DD group, there was a progressive increase in rate (reflecting body radioactive iron turnover) in the following order: D3S, NN, N3S, D1S and N1S.

## DISCUSSION

The present study illustrates the very rapid decrease in true iron absorption (%) that takes place when a daily iron supplement equivalent to 10 times the normal intake is administered preceding two

TABLE 4

True iron absorption in rats by experimental group, day of experiment and day of supplementation<sup>1</sup>

Day of experiment	Day of supplementation	Group <sup>2</sup>					
		NN	DD	N1S	N3S	D1S	D3S
	1	33.1 ± 2.4 <sup>b</sup> (n = 10)	90.3 ± 0.8 <sup>a</sup> (n = 10)	10.1 ± 0.9 <sup>d</sup> (n = 10)	10.1 ± 0.9 <sup>d</sup> (n = 10)	22.6 ± 1.9 <sup>c</sup> (n = 10)	22.6 ± 1.9 <sup>c</sup> (n = 10)
	2			7.6 <sup>b</sup>		12.8 <sup>a</sup>	
	3	36.0 ± 3.9 <sup>b</sup> (n = 6)	86.2 ± 0.9 <sup>a</sup> (n = 6)	8.7, 6.6 (n = 2)		13.5, 12.2 (n = 2)	
	4			9.5 ± 1.4 <sup>c</sup> (n = 8)	9.8	11.8 ± 1.1 <sup>c</sup> (n = 8)	19.4
	7	33.4 ± 1.6 <sup>b</sup> (n = 8)	94.4 ± 2.2 <sup>a</sup> (n = 8)	8.2 (n = 2)	10.0, 6.4 (n = 2)	6.9 (n = 2)	20.5, 18.4 (n = 2)
	8	34.2 ± 3.7 <sup>b</sup> (n = 6)	90.7 ± 4.7 <sup>a</sup> (n = 6)	4.8 ± 0.2 <sup>d</sup> (n = 4)	8.3 ± 1.2 <sup>cd</sup> (n = 4)	5.6 ± 1.4 <sup>d</sup> (n = 4)	13.0 ± 1.4 <sup>c</sup> (n = 4)
	10			7.4 ± 1.0 <sup>c</sup> (n = 8)		7.9 ± 0.6 <sup>c</sup> (n = 8)	
	13			6.6 (n = 2)	10.2 (n = 2)	6.7 (n = 2)	12.4 (n = 2)
				7.7, 5.5 (n = 2)	12.1, 8.3 (n = 2)	7.9, 5.5 (n = 2)	14.0, 10.9 (n = 2)
				4.9 ± 0.7 <sup>b</sup> (n = 4)	9.1 ± 0.4 <sup>a</sup> (n = 4)	5.1 ± 1.0 <sup>ab</sup> (n = 4)	8.2 ± 0.8 <sup>ab</sup> (n = 4)

<sup>1</sup>Values are means ± SE; mean and individual values are given when n = 2. Values with an asterisk represent joint data from the two experiments because the values did not differ from each other. Values on the same study day that do not share a common superscript letter are statistically different ( $P < 0.05$ ).

<sup>2</sup>Group abbreviations: NN, rats kept iron-normal throughout the study (un-supplemented); DD, rats kept iron-deficient throughout the study (un-supplemented); N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study.

HK

VITTI ET AL.

## IRON ABSORPTION BY SUPPLEMENTED RATS

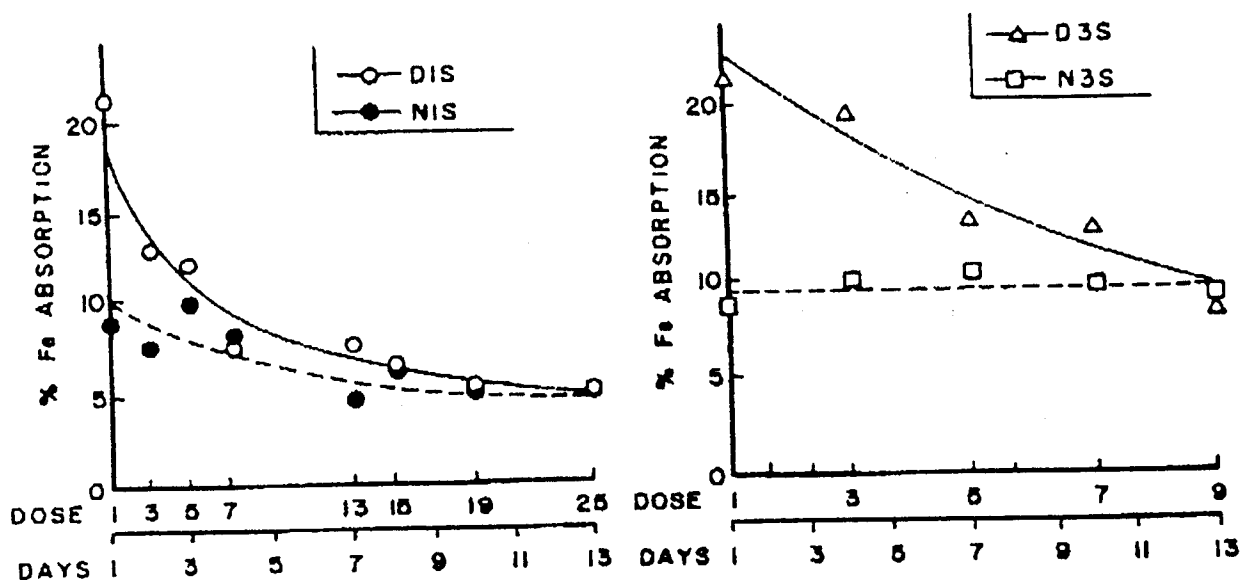


FIGURE 2 True iron absorption (%) of supplemental iron in meal fed iron normal (N groups) and non-deficient rats (D groups) receiving 10 times the "usual" iron intake as a supplement in two premeals every day (NIS and DIS) or every 3 d (N3S and D3S). Each point represents the mean true iron absorption of the specified supplemental iron doses. Days refer to days of supplementation. Please see Table 4 for error terms at each point.

meals in rats trained to meal feed twice daily. This marked and rapid decrease is observed in both iron-normal and iron-deficient rats, particularly in the latter. There are no similar detailed studies in absorption of subsequent iron supplemental doses in

experimental animals or humans. These results expand the present knowledge on the blocking effect of previous high iron intake and the effect of iron repletion on iron absorption and possibly provide some basis for revision of common supplementation practices in humans.

The experimental design and iron supplementation dose were chosen to simulate the present recommendations for treatment and universal iron supplementation in pregnant women (Chatoenlap et al. 1988, DeMaeyer 1989), which consist of the daily intake of 120-240 mg of elemental iron as  $FeSO_4 \cdot H_2O$ , in two to four doses of 60 mg of Fe each, preferably before meals or a few hours after meals. These doses represent about 10 times the usual food iron intake of women of reproductive age (Food and Agricultural Organization of the United Nations 1988).

Rapidly growing male rats, iron-deficient or iron-normal, were chosen as a model to simulate universal supplementation of iron-deficient or normal women, because, even though we recognize that the rapidly growing rat relies more on dietary iron than on recycling of internal iron for their iron economy, the rat is a well-accepted animal model for studying absorption and utilization of dietary and supplemental doses of nonheme iron (Annie and Hegsted 1971).

The experimental model was successful in inducing iron deficiency, in maintaining "normal" iron nutrition (in groups DD and NN, respectively) and in providing the supplemented groups the desired amount of iron in a premeal. Group DD became

TABLE 5

Iron rates of loss in iron-normal and iron-deficient rats without and with daily and every-3-d supplementation<sup>1</sup>

Group	No. of measurements	Iron loss per day	
NN	30	0.097 <sup>a</sup>	0.011
DD	30	0.039 <sup>b</sup>	0.004
NDV	30	0.399 <sup>c</sup>	0.019
N3S	30	0.130 <sup>b</sup>	0.012
D3S	30	0.139 <sup>b</sup>	0.016
D3S	30	0.083 <sup>b</sup>	0.016

<sup>1</sup>Values are means (s.e.). Values with different superscripts in different rows are different ( $P < 0.05$ ).

Group abbreviations: NN, rats kept iron-normal throughout the study (nosupplemented); DD, rats kept iron-deficient throughout the study (nosupplemented); N3S, iron-normal rats receiving daily iron supplementation starting on d 13 of the study; D3S, iron-deficient rats receiving daily iron supplementation starting on d 13 of the study; NDV, iron-normal rats receiving iron supplementation every 3 d starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d starting on d 13 of the study.

## TRUE ABSORPTION OF SUPPLEMENTAL IRON

89

progressively more anemic in the course of the study, at a rate similar to that reported by Dallman et al. (1982) in severely iron-deficient rats. Iron-deficient supplemented rats (D1S and D3S groups) replenished their lower hemoglobin concentrations, improved their food intake and growth rates, and presented no adverse clinical evidence caused by the supplement. Iron-normal supplemented rats (N1S and N3S groups) maintained their hemoglobin values, food intakes and growth rates and presented no evident clinical deterioration, although premeal intake appeared to be more difficult in the daily supplemented groups. Growth rates in the NN, N1S and N3S groups were, on average, 88% of rates for normal growing male Prague-Dawley rats given free access to a complete diet (Rogers 1979). Therefore, meal-feeding twice daily had a small effect on growth among iron-normal animals, and iron supplementation did not have an appreciable effect on food intake.

Percent iron absorption was, as expected, highest in the DD group receiving only a trace of  $^{59}\text{Fe}$ . It remained >86% throughout the period of study. Iron absorption was similarly stable (mean level of 34%) in the NN groups, indicating a steady level of iron nutrition throughout the study. In the daily supplemented groups a rapid decline in percent iron absorption was evident, particularly in the D1S groups, which by the third day (dose 5) reached a level similar to that in the N1S group. This last group showed a progressive decline in percent iron absorption, which, as expected began at a lower level than in the D supplemented groups. Both daily supplemented groups (N1S and D1S) presented a stable iron absorption after the seventh iron supplement dose, which oscillated around an average 7.2%. The groups supplemented every 3 d present a different picture. The D3S group maintained higher percent absorption that declined slowly, reaching levels similar to those of the other supplemented groups only on d 13 (dose 9). The N3S group maintained a stable level of iron absorption around a mean of 9.5%, which was significantly different from the joint data obtained in the latter part of the study among the daily supplemented groups. Both groups supplemented every 3 d absorbed more iron than the daily supplemented groups by d 13 due to a further decline in percent absorption in the latter groups.

When percent iron absorption was estimated in groups D1S and D3S at four points where cumulative iron absorption (mg) was the same in both groups, the former group absorbed  $0.53 \pm 0.06$  (mean  $\pm$  SD) the percent absorption of D3S. This can be interpreted as the magnitude of the "absorption blockage." There was no clear tendency for this percent difference in iron absorption to change as time (and cumulative iron absorption) increased.

Daily rates of radioactive iron loss for D1S and N1S are close to two and four times, respectively, the rates

observed in the NN group. On the other hand, the rates of loss among groups supplemented every 3 d (N3S and D3S) are no different from those of the NN group. We interpret these results as indicating that intermittent iron supplementation is better than daily supplementation in two aspects: iron absorption is more efficient and radioactive iron turnover remains close to the values found in "normal" rats. The significantly higher daily iron loss observed in the daily iron supplemented groups also suggests alterations in total body iron metabolism. This assertion is supported by iron distribution studies of these rats, which have been partially reported in abstract form (Martin et al. 1990 and unpublished data).

These results indicate that, by providing iron supplements in an intermittent dosage tailored to intestinal mucosal turnover rates and aimed at avoiding iron absorption blockage caused by maintaining an iron-saturated environment in the gut, the efficiency of iron absorption and retention can be markedly improved. This is illustrated by comparing total absorbed iron (mg) throughout the supplementation period studied (Table 6). The greater efficiency of utilization of supplementation every 3 d over that of daily iron supplementation is evident: 1) Supplemental iron intake in 13 d in the N3S group was 38% of that ingested in the same period by group N1S, yet total absorbed iron in the former group was 60% that of the latter (3.64/6.02 mg). Group N3S ingested a normal iron-containing premeal (400  $\mu\text{g}$ ) twice daily the two days between supplemental doses.

TABLE 6

Mean relative efficiency of total supplemental iron absorbed in 13 d after the beginning of supplementation

Group <sup>1</sup>	Iron intake	Absorbed iron	Efficiency of absorption <sup>2</sup>
	mg	mg	%
N1S	99.53	6.02	6.05
N3S	44.40 <sup>3</sup>	3.64 <sup>4</sup>	9.51 <sup>4</sup>
D1S	99.53	8.13	8.17
D3S	38.28	6.96	18.18

<sup>1</sup>Group abbreviations: N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d starting on d 13 of the study; D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study.

<sup>2</sup>Efficiency of absorption = (absorbed iron/iron intake)  $\times$  100.

<sup>3</sup>Includes 38.28 mg from supplemented premeal and 6.12 mg from the premeal with "normal" iron content consumed the days between supplementation.

<sup>4</sup>Total milligrams and percentages of supplemental iron only. Absorption of dietary iron for the 2 d between supplementation doses is assumed to be 0.

Therefore the total iron intake of this group in 13 d amounts to 44.4 mg of iron, or 45% of that in group N1S. The absorption of the iron ingested between supplemented doses was not measured. The total absorbed iron in 13 d by group N3S estimated only on the basis of percent absorption of supplemental iron assumes 0% iron absorption in the days between supplements and is therefore a conservative estimate of total iron absorption. 2) Even though in the D3S group supplemental iron intake in 13 d was 38% that in the D1S group, total absorbed iron was 85% that of the latter group (6.96/8.13 mg). 3) Overall efficiency of iron absorption in 13 d of supplementation is 1.57 and 2.22 times greater for N3S and D3S groups than that of their counterparts receiving daily iron supplementation (9.51/6.05% and 18.18/8.17%, respectively). Wright and Southon (1990) have also shown that iron supplementation every 2 or 3 d (four times the normal intake) supplied as added iron in the meals fed to iron-deficient rats for a total of 7 d is more efficiently utilized than daily supplemental iron, based on hematological variables and liver iron contents. Relative efficiency of iron utilization is difficult to estimate from their data.

The mean relative efficiency of true iron retention in 13 d, estimated from the cumulative iron absorption minus that due to cumulative iron losses because of iron turnover rates (Table 6), is slightly more favorable for the every-3-d dosage: relative efficiencies are 0.62 and 1.60 for N3S and 0.86 and 2.25 for D3S for percent retained and efficiency ratio, respectively, in relation to those of their daily supplemented counterparts (N1S and D1S).

It is obvious that as supplementation periods are lengthened the "advantage" of every-3-d supplementation over daily supplementation disappears for repletion of iron deficiency. However, by then iron repletion has occurred in the groups supplemented every 3 d, and the advantage of avoiding excess retained iron by intermittent dosing becomes important.

This aspect of the data is presented in Table 7 as excess iron absorbed and retained by the supplemented groups above that absorbed and retained by the normal animals (NN group). It is clear that iron supplementation every 3 d vs. daily iron supplementation reduces the risk of iron overload among iron-normal rats. Iron overload, even if temporary and more localized to the intestine and the liver, may have negative consequences in terms of symptoms and through promoting free radical-induced oxidative processes (Gutteridge 1990). In effect, the N1S group accumulated 74% excess iron in relation to the NN's retained iron (2.50 mg excess over 3.38 mg normal retention), and 10.4 times more excess iron than the N3S group. This last group retained only 7% excess supplementary iron compared with the NN group. It is possible that radioactive iron rates of loss are an indication of a condition of temporary iron overload. The fact that the rates of loss do not increase as excess iron increases with time in group N1S strongly

TABLE 7

Mean total excess iron absorbed and retained by rats in 13 d of supplementation by the different supplemented groups in relation to the iron-normal group

Group <sup>1</sup>	Total absorbed	Total retained	Excess absorbed	Excess retained
	mg			
NN	3.40 <sup>2</sup>	3.38 <sup>3</sup>	—	—
N1S	6.02	5.88	2.62 <sup>a</sup>	2.50
N3S	3.64	3.62	0.24	0.24
D1S	8.13	8.02	4.73	4.64
D3S	6.96	6.94	3.56	3.56

<sup>1</sup>Group abbreviations: NN, rats kept iron-normal throughout the study (unsupplemented); DD, rats kept iron-deficient throughout the study (unsupplemented); N1S, iron-normal rats receiving daily iron supplementation, starting on d 13 of the study; D1S, iron-deficient rats receiving daily iron supplementation, starting on d 13 of the study; N3S, iron-normal rats receiving iron supplementation every 3 d, starting on d 13 of the study; D3S, iron-deficient rats receiving iron supplementation every 3 d, starting on d 13 of the study.

<sup>2</sup>Each value is the mean cumulative iron absorbed by each group from d 1 to d 13 of supplementation.

<sup>3</sup>Each value is the mean cumulative iron absorbed corrected by the mean iron rate of iron loss for each group during the 13 d of supplementation.

suggests that there is no direct quantitative relationship between these two variables.

Extrapolating to the case of humans, although this must be done with caution, the few studies on sequential iron absorption of 100-mg supplemental or therapeutic iron doses administered to iron-deficient subjects reveal that in the first 10 d mean retention averaged 16.6%, followed by 11% and 8% after 30 and 40 d of supplemental intake (Norrby and Solvell 1974). These studies clearly show the inefficiency of these therapeutic iron schemes, which leave up to 92% of the ingested iron unabsorbed and remaining in the intestinal lumen and in the mucosal cells. This prolonged "localized iron overload" is far from physiologically desirable. Moreover, the average of 16.6% absorption in the first 10 d of iron supplementation could be the result of a high absorption rate in the first few days that fell rapidly, in a fashion similar to what we have shown in rats, to a stable level of 8% as treatment is prolonged (similar to that seen in our studies from d 5 to 13 of supplementation). On the basis of these findings, iron supplementation studies in humans comparing daily, twice weekly and every week are in progress, considering the 5-6-d turnover time of intestinal cells in humans (Lipkin 1981).

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## TRUE ABSORPTION OF SUPPLEMENTAL IRON

91

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