Effects of Stearic Acid and Beef Tallow on Iron Utilization by the Rat (43457)

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Abstract. **Two experiments were done in which anemic rats were fed diets containing safflower oil or stearic acid and low (10 ppm) or adequate (39–42 ppm) iron. Diets were 24% fat by weight. In the stearic acid diets, 2% (Experiment 1) or 4% (Experiment 2) of the fat was supplied by safflower oil to satisfy essential fatty acid requirements. Repletion of hemoglobin, hematocrit, and liver iron was assessed. Compared with safflower oil in both experiments, stearic acid had a significant positive effect ($P < 0.0001$) on repletion of hemoglobin (Hb), hematocrit (Hct), and liver iron concentration; the effect on Hb and Hct was most pronounced when dietary iron was low. When expressed as g Hb/mg Fe intake, Hb repletion was affected by a significant interaction between fat and Fe ($P < 0.002$) and was greatest in rats fed low iron stearic acid diets. In a third experiment, rats were fed low dietary iron and 24% safflower oil, 20% stearic acid + 4% safflower oil, 3.2% stearic acid + 20.8% safflower oil, or 20% beef tallow + 4% safflower oil. The 20% beef tallow provided 3.2% stearic acid in the total diet. The responses of Hb and Hct were similar to those in the first two experiments for rats fed safflower oil or stearic acid. Rats fed beef tallow had significantly greater ($P < 0.05$) Hb and Hct repletion than did rats fed safflower oil, although the degree of repletion was less than that observed in rats fed 20% stearic acid. There was no difference in iron repletion of rats fed 3.2% stearic acid and rats fed beef tallow. We conclude that stearic acid enhances iron utilization by rats.** [P.S.E.B.M. 1992, Vol 200]

There is evidence that the amount of dietary fat and its degree of saturation can affect the utilization of dietary iron. Bowering *et al.* (1) and Amine and Hegsted (2) found that increasing dietary fat or changing to a more saturated fat source increased iron absorption. The development of iron deficiency in rats was promoted by the feeding of unsaturated fat (3–5) and retarded by the feeding of fat-free or saturated fat diets (3, 4). We reported (6) that rats fed coconut oil had higher hemoglobin and higher liver iron than rats fed safflower oil; similar effects were found when dietary fat (either coconut or safflower oil) was increased from 5% to 30%. Lukaski *et al.* (7) found that athletes fed diets high in saturated fat had a much more positive iron balance than when they were fed otherwise identical diets high in polyunsaturated fats or low in fat. Van Dokkum *et al.* (8) reported a decreased iron balance in humans when linoleic acid intake was increased from 4% to 16% of energy in a diet containing 42% of energy as fat.

Meat has also been found to affect the absorption of nonheme iron. Efforts to identify the "meat factor" have been extensive (9–14). Several authors have concluded that the active factor in meat that promotes nonheme iron absorption is an amino acid such as cysteine or histidine, or small peptides such as glutathione, but the effects of these and other components of meat on iron absorption have not always been consistent. In a recent review, Zhang *et al.* (14) suggested that the meat factor must be something which binds iron, but that some mechanism other than amino acid or peptide chelation is operative.

Mahoney *et al.* (15) reported a relationship between the fat effect and the meat effect on iron absorption. They found that, compared with those fed turkey fat, corn oil, or pork fat, animals fed beef fat were most efficient at incorporating iron from turkey meat into hemoglobin. One of the principal differences between beef fat and the other fats that Mahoney *et al.* (15) used is that beef fat contains approximately 19% stearic acid

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Table I. Composition of Iron-Deficient and Experimental Diets[a]

Component	Experiment 1					Experiment 2				Experiment 3			
	Fe-Defic.	Stearic-Low Fe	Stearic-High Fe	Saff-Low Fe	Saff-High Fe	Stearic-Low Fe	Stearic-High Fe	Saff-Low Fe	Saff-High Fe	Stearic-20%	Saff-24%	Stearic-3.2%	Tallow 20%
Casein, vitamin-free	200	200	200	200	200	200	200	200	200	200	200	200	200
Sucrose	450	330	330	330	330	310	310	310	310	310	310	310	310
Cornstarch	150	147.5	135	147.5	135	147.5	135	147.5	135	147.5	147.5	147.5	147.5
Cellulose	50	50	50	50	50	50	50	50	50	50	50	50	50
Safflower oil	100	20	20	220	220	40	40	240	240	40	240	208	40
Stearic acid	0	200	200	0	0	200	200	0	0	200	0	32	0
Beef tallow	0	0	0	0	0	0	0	0	0	0	0	0	200
DL-Methionine	3	3	3	3	3	3	3	3	3	3	3	3	3
Choline Cl	2	2	2	2	2	2	2	2	2	2	2	2	2
AIN-76A vitamin mix	10	10	10	10	10	10	10	10	10	10	10	10	10
Mineral mix (-Fe)[a]	35	35	35	35	35	35	35	35	35	35	35	35	35
Iron mix[b]	0	2.5	15	2.5	15	2.5	15	2.5	15	2.5	2.5	2.5	2.5

[a] Data are expressed as g/kg.
[b] AIN-76 mineral mix without iron.
[c] Cornstarch plus iron as ferric citrate to provide 2 mg Fe/g mix.

(18:0), which is 10 times higher than the stearic acid content of corn oil, three times the stearic acid content of turkey fat, and 1.5 times greater than that in lard (16). Generally, the type of beef used in studies of iron absorption has not been reported, but even the "lean" ground beef available at groceries contains about 15% fat. We thought it might be possible that beef fat was responsible for part of the enhancement of iron absorption by beef.

We report here the results of experiments designed to evaluate the effect of stearic acid on iron utilization.

Materials and Methods

Animals and Diets. *Experiment 1.* Forty male weanling Sprague-Dawley rats were obtained from Harlan Sprague Dawley, Indianapolis, IN.[2] The animals were house individually in stainless steel wire cages in a temperature-controlled room with 12-hr cycles of light and dark. Animals (mean weight, 59.1 ± 7.5 g, 7 ± SD) were fed an iron-deficient diet containing 5 mg iron/kg and 10% safflower oil (Table I) *ad libitum* for approximately 4 weeks. They were then divided into five groups of eight animals each with equal mean hemoglobin values. One group of rats was sacrificed and blood and liver were taken for analysis, as described below; hemoglobin concentration in the cardiac blood of these animals was 5.7 ± 0.2 g/dl. The other four groups were fed experimental diets containing either 22% safflower oil or 20% stearic acid and 2% safflower oil, and containing iron concentrations of either 10–11 mg/kg or 39–42 mg iron/kg (Table I) in a 2 × 2 factorial design. Hemoglobin values in tail vein blood for the four groups were 7.1 ± 0.7 g/dl, 7.0 ± 0.7 g/dl, 7.1 ± 0.7 g/dl, and 7.0 ± 0.8 g/dl for safflower-low, safflower-high, stearate-low, and stearate-high iron, respectively. The weights of the rats at the beginning of iron repletion were 98 ± 7 g, 89 ± 11 g, 90 ± 12 g, and 95 ± 15 g, respectively. All rats in the four experimental groups were pair fed to prevent differences in food intake among groups. Rats in the low Fe-safflower oil group served as the control group by which food given to rats in other experimental groups was determined. The diet formulation was based on that recommended by the American Institute of Nutrition (AIN) (17), except that dietary fat was higher and dietary iron content varied. The increase in fat content from that recommended by American Institute of Nutrition was compensated for by decreases in sugar and starch content. Diets were stored frozen at −20°C to prevent oxidation and fresh food was given daily. Because of the limited amount of food offered, food consumption was always >95% of the amount offered; thus, food did not remain in food cups with the chance of oxidative damage occurring.

Experiment 2. Experiment 2 was similar to Experiment 1, except that diets contained 24% safflower oil or 20% stearic acid plus 4% safflower oil. More safflower oil was added to the stearic acid diet than in Experiment 1 in an effort to correct poor growth by animals fed stearic acid (see below). Except for the diet composition, the two experiments were identical. The mean weight of rats (eight per group) at the beginning of the experiment was 42.5 ± 7.5 g. Hemoglobin in tail vein blood before beginning iron repletion was 7.1 ± 0.6 g/dl, 7.0 ± 0.9 g/dl, 7.1 ± 0.7 g/dl, and 7.1 ± 0.7 g/dl for safflower-low, safflower-high, stearate-low, and stearate-high iron, respectively. Weights at the beginning of repletion were 75 ± 8 g, 76 ± 6 g, 70 ± 8 g, and 70 ± 9 g, respectively.

Experiment 3. Experiment 3 was designed to examine the effect of feeding a practical source of stearic

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

acid, beef tallow, on iron bioavailability. After iron depletion by diet, rats were fed one of four diets, all containing 10–11 mg iron/kg. Iron-adequate diets were not used in this experiment because the greatest relative effects of dietary fat in previous experiments were found in rats fed low iron. One diet contained 24% safflower oil as the dietary fat, one contained 20% stearic acid and 4% safflower oil, one contained 20% beef tallow (Interstate Foods, Chicago, IL) and 4% safflower oil, and the fourth contained 3.2% stearic acid (the amount of stearic acid contained in the 20% tallow diet) and 20.8% safflower oil (Table I). Experimental procedures were identical to those used in the first two experiments. The beef tallow contained 16% stearic acid, according to gas chromatographic analysis (analysis furnished by supplier). The mean weight of rats (eight per group) at the beginning of the experiment was 47.5 ± 6.2 g. Hemoglobin values in tail vein blood were 7.8 ± 1.3 g/dl, 7.5 ± 1.1 g/dl, 7.6 ± 0.7 g/dl, and 7.5 ± 1.1 g/dl for safflower, stearate, stearate + safflower, and tallow groups, respectively. The weights of the four groups of rats at the beginning of iron repletion were 85 ± 6 g, 84 ± 7 g, 87 ± 11 g, and 81 ± 8 g, respectively.

Biochemical Measurements. After they had consumed the experimental diets for a total of 5 weeks, the rats were fasted overnight and then sacrificed under sodium pentobarbital anesthesia by cardiac exsanguination with a heparin-coated needle and syringe. Livers were removed for iron analysis. Hemoglobin, hematocrit, plasma iron, and liver iron were determined. Hemoglobin was measured by a Coulter Counter model S+4 (Coulter Electronics, Hialeah, FL). Freeze-dried liver was wet digested with nitric acid and hydrogen peroxide (18) and iron in liver and plasma was measured by atomic absorption spectroscopy (Perkin Elmer 503; Perkin Elmer, Norwalk, CT). Replicate analyses of National Institute of Standards and Technology bovine liver (SRM 1577a) yielded values of 201 ± 3 μg/g in Experiment 1, 215 ± 10 μg/g in Experiment 2, and 209 ± 10 μg/g in Experiment 3; the certified value is 194 ± 20 μg/g (mean ± SD). The increase in hemoglobin per milligram of iron consumed was calculated from changes in total body hemoglobin at the beginning and end of the iron repletion period (19) using a blood volume of 64.7 ml/kg body wt (20). This procedure corrects for differences in body weight among animals (19).

Data were analyzed by analysis of variance (ANOVA) (21) and Tukey's (with one-way ANOVA) or Scheffé (with two-way ANOVA) contrasts.

Results

Experiment 1. Food intake was not affected by dietary treatment because of pair feeding, but the final weight of rats was significantly affected by dietary fat (Table II). There was a tendency for dietary iron to also affect body weight. Rats fed safflower oil were significantly ($P < 0.0001$) heavier than rats fed stearic acid; rats fed the adequate iron (35 ppm) diets tended to be heavier than those fed diets low in iron ($P < 0.08$).

Final hemoglobin and hematocrit were significantly affected by both dietary iron and dietary fat (Table II) and by an interaction between iron and fat such that the positive effect of stearic acid on hemoglobin and hematocrit was most pronounced in rats fed the low iron diets. Both dietary iron and fat affected plasma iron, although the effect of fat on plasma iron was only marginally significant. Total liver iron and liver iron concentration were affected by dietary fat in rats fed adequate iron but not in rats fed low iron. In general, values of indices of iron status in blood were higher in rats fed stearic acid than in rats fed safflower oil, and the effects of stearic acid on blood indices were greatest in rats fed low iron diets, but effects of stearic acid on liver iron were greatest in rats fed adequate iron. When hemoglobin synthesized per milligram of iron intake was calculated, a significant iron × fat interaction ($P = 0.001$) was noted. Thus, the enhancement of iron utilization by stearic acid was observed at low levels of dietary iron intake using this criterion, but at the higher level of dietary iron, the effect was reversed.

Experiment 2. Food intake was not affected by dietary treatment because of pair feeding, but the final weight of rats was significantly affected by both dietary fat and dietary iron (Table II). The feeding of stearic acid, which resulted in lower weights, had a more pronounced effect on weight than did the concentration of dietary iron.

Final hemoglobin and hematocrit were significantly affected by both dietary iron and dietary fat (Table II) and by an interaction between iron and fat such that the positive effect of stearic acid on hemoglobin and hematocrit was most pronounced in rats fed the low iron diets. Liver iron concentration was also affected by both dietary iron and fat, but the differences were significant only in rats fed high iron diets. Total liver iron was significantly affected only by dietary iron, but there was, again, a tendency for stearic acid to have a positive effect on liver iron in rats fed adequate iron. In general, values of iron status indices were higher in rats fed stearic acid than in rats fed safflower oil; the effects of stearic acid on hematologic indices were greatest in rats fed low iron diets, whereas effects of stearic acid on liver iron were greatest in rats fed adequate iron. In this experiment, as in Experiment 1, a significant iron × fat interaction on g Hb/mg Fe intake was found. At low levels of dietary iron intake, hemoglobin synthesis was more efficient in rats fed stearic acid than in rats fed safflower oil. At the higher level of iron intake, the two fats did not differ in their effects.

Experiment 3. Responses of hemoglobin and hem-

Table II. Iron Status Indices in Rats Fed Stearic Acid or Safflower Oil and Low or Adequate Dietary Iron[a]

Dietary fat	Dietary Fe[b] (mg/kg)	Final wt (g)	Hb (g/dl)	Hct (%)	Plasma Fe (μg/ml)	Liver Fe (μg/g)	Total liver Fe (μg)	Food intake (g/d)	g Hb/mg Fe intake
				Experiment 1					
Safflower oil	10.4	255	6.9*	20.9*	0.49	112*	294*	12.7	0.145*
Stearic acid	11.4	187	11.6‡	34.7‡	0.78	145*	257*	12.1	0.192†
Safflower oil	38.7	267	13.9†	41.8†	2.48	218†	581†	12.6	0.112‡
Stearic acid	41.9	206	15.1§	45.3§	2.86	374‡	821‡	13.0	0.082§
Root mean square error		25	0.6	1.9	0.47	28	90	1.3	0.013
ANOVA (*P*-values)									
Fat		0.0001	0.0001	0.0001	0.06	0.0001	0.003	NS	NS
Fe		0.08	0.0001	0.0001	0.0001	0.0001	0.0001	NS	0.0001
Fat × Fe interaction		NS	0.0001	0.0001	NS	0.0001	0.0002	NS	0.0001
				Experiment 2					
Safflower oil	9.2	220	5.6†	18.6†	0.59	116†	264	11.2	0.153†,‡
Stearic acid	9.1	152	8.4§	26.1§	0.69	159†	242	10.4	0.185‡
Safflower oil	37.7	234	12.9‡	37.2‡	2.01	252‡	614	11.1	0.133*,†
Stearic acid	37.6	171	13.6‡	39.0‡	1.96	368§	682	11.7	0.098*
Root mean square error		21	0.6	2.1	0.39	33	99	1.5	0.027
ANOVA (*P*-values)									
Fat		0.0001	0.0001	0.0001	NS	0.0001	NS	NS	NS
Fe		0.04	0.0001	0.0001	0.0001	0.0001	0.0001	NS	0.0001
Fat × Fe interaction		NS	0.0001	0.0008	NS	0.005	NS	NS	0.002

[a] Values in the same column with different symbols (*, †, ‡, §) are significantly different from one another ($P < 0.05$) by Scheffé contrasts.
[b] Analyzed values.

atocrit were similar to those observed in the first two experiments for the rats fed safflower oil or stearic acid (Table III). Rats fed beef tallow had a significantly ($P < 0.05$) greater hemoglobin and hematocrit response than did rats fed safflower oil, although the degree of repletion was less than that observed when stearic acid was the principal dietary fat. The utilization of iron in rats fed only 3.2% stearic acid was identical to that of rats fed beef tallow, based on hematologic data, and significantly greater than iron utilization in rats fed safflower oil. Liver iron concentrations were lower in rats fed safflower oil than in rats fed the other three diets. However, because rats fed 20% stearic acid did not grow as well as rats fed safflower oil, tallow, or the 3.2% stearic acid diet, total liver iron was lowest in rats fed 20% stearic acid. Efficiency of dietary iron incor-

Table III. Iron Status Indices in Rats Fed Stearic Acid, Safflower Oil, or Beef Tallow, with Low Iron[a]

Dietary fat[b]	Experiment 3								
	Dietary Fe[c] (mg/kg)	Final wt (g)	Hb (g/dl)	Hct (%)	Plasma Fe (μg/ml)	Liver Fe (μg/g)	Total liver Fe (μg)	Food intake (g/d)	g Hb/mg Fe intake
Safflower oil	9.2	220*	5.6*	18.1*	0.45	82.2*	234*	11.2	0.113
Stearic acid[d]	10.8	155†	8.2†	25.9†	0.53	118.6†	188†	10.7	0.114
Stearic/saff.[e]	10.9	211*	6.8‡	21.6‡	0.44	96.4‡	225*,†	10.4	0.139
Beef tallow[f]	10.7	204*	6.9‡	21.5‡	0.47	104.2‡	208*,†	11.0	0.141
Root mean square error		21	0.7	2.1	0.14	9.4	28	1.3	0.031
ANOVA									
Fat		0.0001	0.0001	0.0001	NS	0.0001	0.02	NS	NS

[a] Values in same column with different symbols (*, †, ‡) are significantly different ($P < 0.05$) by Tukey's studentized range test.
[b] Total dietary fat in all diets was 24%.
[c] Analyzed values.
[d] Twenty percent stearic acid and 4% safflower oil to meet essential fatty acid requirements.
[e] Stearic acid (3.2%) and safflower oil (20.8%).
[f] Diet contained 3.2% stearic acid provided by tallow.

poration into hemoglobin was not affected by dietary fat in this experiment.

Discussion

This study confirms previous observations (1, 2, 6) that saturated fat enhances nonheme iron utilization in the rat. In this study, as well as in other work, the effect was most pronounced when dietary iron was low or marginal.

In earlier work (6), we found similar effects in the rat, but the results were somewhat more difficult to interpret because of differences in food intake by rats fed different amounts and sources of fat. In these studies, we held food intake constant by pair feeding all rats to avoid the possible confounding effects of variations in food or iron intake.

In the first experiment, we observed unexpectedly depressed body weights in the rats fed stearic acid, although total food intake did not differ between rats fed stearic acid or safflower oil. Although the stearic acid diets also contained 2% safflower oil, which should have satisfied the requirement for essential fatty acids (22), it seems possible that the essential fatty acid requirement might be higher in rats fed a high proportion of saturated fat (23–26). However, when we doubled the proportion of added safflower oil to 4% of the diet in Experiment 2, the weight differences persisted between the groups fed safflower oil and stearic acid. Evidently, the poor weight gain in rats fed high amounts of saturated fat is not caused by an increased requirement for essential fatty acids, but by some change in energy metabolism when diets are high in saturated fat (27). Although we did not succeed in resolving the problem of poor weight gain in rats fed stearic acid in these experiments, the changes in iron status were consistent in both experiments. Furthermore, when we calculated the amount of hemoglobin synthesized per milligram of iron ingested, a procedure that corrects for differences in body weight, the effects of stearic acid persisted, and they were greatest when iron intake was low. Poor digestibility of stearic acid could be a cause of the lower weight gain by the animals fed stearic acid. However, studies that showed poor weight gain in animals fed stearic acid used stearic acid fed in the form of triglycerides (28–30). Stearic acid from tristearin is less well absorbed than di- or monostearin (28). However, we fed stearic acid as a free fatty acid. There is no evidence to suggest that stearic acid is less well absorbed in this form than other fatty acids (31). Thus, we do not believe that the differences in weight gain in the experiments were related to poor digestibility or absorption of stearic acid.

In the third experiment, we fed the rats a practical source of stearic acid, beef tallow. Beef tallow usually contains 18–20% stearic acid, so that the tallow diet had a much lower stearic acid content (3.2%) than did the diets in the first two experiments. Nevertheless, there was a significant effect on hemoglobin, hematocrit, and liver iron concentration when beef tallow was fed, compared with diets using safflower oil as the only fat source. The effect of the tallow diet was virtually identical to that observed when 3.2% stearic acid and 20.8% safflower oil was fed, which suggests that the effect of feeding tallow was related to its stearic acid content.

No significant effects of dietary fat on efficiency of hemoglobin synthesis from dietary iron (g Hb/mg Fe intake) were observed in the third experiment. However, the two groups fed beef tallow and stearic acid plus safflower oil did have higher values for this parameter than the group fed only safflower oil. Variability among animals in the stearic acid group was somewhat higher than for the other groups. Two animals in the stearic acid group had much lower values (0.064 ± 0.019 g Hb/mg Fe intake) than the other six animals in the group (0.131 ± 0.027 g Hb/mg Fe intake), so a tendency for enhancement of iron utilization by stearic acid was present.

The fact that the difference between the hemoglobin repletion rates (g Hb/mg Fe intake as well as final g Hb) of stearic acid and safflower-fed animals was greater when they were fed low iron diets than when they were fed adequate iron suggests that enhancement of iron availability by stearic acid is most important when dietary iron is limiting. When dietary iron was adequate, the amount of iron absorbed from either stearic or safflower oil diets was evidently sufficient to support near maximal rates of erythropoiesis. The effect of stearic acid on liver iron was most pronounced when dietary iron was adequate, and suggests that the iron supply for erythropoiesis was not limiting, and iron was available for storage; thus, the availability of iron for storage was enhanced by stearic acid.

Stearic acid has been suggested to affect iron absorption at the cellular level. Stearic acid in cigarette smoke increases iron uptake by pulmonary macrophages (32). It was demonstrated by using an *in vitro* brush-border membrane preparation that the major iron-binding components were associated with free fatty acids and that oleic and stearic acids showed iron-binding capacities (33, 34). Such enhancement of iron absorption by stearic acid may be related to the formation of stable monolayer stearate-iron films (35). Monolayer films of lipid anions may function as ionophores in the translocation of cations across biological membranes (36).

There are a number of differences in iron utilization between rats and humans. In particular, rats absorb relatively less heme iron than nonheme iron (37), which is the opposite of the relationship found in humans, and the total percentage of iron absorbed is higher in rats than in humans. Nevertheless, a significant corre-

lation has been noted between nonheme iron availability in the rat and in humans (38). An effect of the so-called meat factor has also been found in rats (39). Thus, we believe that the results of our study provide information that may be pertinent to human nutrition. Furthermore, our results are consistent with data concerning the effects of fat on iron metabolism in humans (7, 8). However, confirmation of these findings in other species, especially humans, should be sought.

The enhancement of iron utilization by stearic acid has obvious implications for human diets, because stearic acid is the principal fatty acid in most meat fats. Reductions in the consumption of animal fats have been recommended both for reducing caloric intakes and for reducing serum cholesterol (40). However, men fed diets for 5 weeks that contained 35% fat, 60% of which was from beef fat, had normal serum cholesterol (41). Bonanome and Grundy (42) compared effects of feeding palmitate, oleate, and stearate on serum cholesterol levels and found that stearic acid decreased serum cholesterol levels. The amount of palmitic acid, the other major fatty acid in meat, which does increase serum cholesterol (43), can be reduced by dietary means or by genetic manipulation in nonruminants (44).

Meat consumption is associated with higher iron status in women (45); this is probably related in part to slightly higher intakes of iron by meat eaters and the higher bioavailability of heme than nonheme iron. We suggest that it might also be related to higher stearic acid consumption by meat eaters than by women who eat predominantly fish and poultry or who are lacto-ovo vegetarians.

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