Effect of iron stores and hysterectomy on iron absorption and distribution in pregnant mice

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Batey, Robert, and Neil Gallagher. Effect of iron stores and hysterectomy on iron absorption and distribution in pregnant mice. Am. J. Physiol. 252(1): E57–E61, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 1(1): E57–E61, 1977. – Intestinal uptake (U) and transport (T) of 55Fe from a 1-μg oral dose was studied in the mouse. U increased to a similar degree in both pregnant and iron-deficient animals. The increase in T was greater in pregnant than in iron-deficient mice. In iron-loaded pregnant mice, U increased to levels found in pregnant animals, whereas T increased, but by a lesser amount. Termination of pregnancy by delivery or hysterectomy at days 20-21 resulted in a fall in U and T to normal levels within 24 h. Eighty percent of iron transported across the intestine in pregnancy was recovered from the fetuses, compared to 67% recovered in the maternal liver. Hepatic uptake was lower in pregnant than in iron-deficient mice or in postpartum mice with persistent iron deficiency. Hepatic uptake by the maternal liver after an intravenous dose of 2 μg 55Fe increased to levels in iron-deficient mice within 5 min of hysterectomy. The results demonstrate an effect of pregnancy on intestinal iron transport and its distribution that was dependent on the presence of fetuses and placentas.

METHODS

Preparation of animals. Sydney white virgin female mice were used throughout the study and were 12–14 wk of age at the time of use. Animals were housed in plastic cages with galvanized wire lids and were fed on a diet of standard rat cubes. Water was allowed ad libitum and all animals were fasted for 16 h prior to study.

Iron deficient mice (FeD) were prepared by weaning animals on an iron-depleted diet of hominy (2 parts), powdered skim milk (1 part) and powdered whey (1 part), to which a vitamin premix (Allied Feeds, Rhodes, South Wales, Australia) was added. Iron overload (FeL) was produced by injecting mice with 5 mg of Imferon (Fisons, P. L., Sydney New South Wales), and iron dextran complex, on four occasions 1 mo before study. Animals were mated at 9–11 wk of age, and gestational age was timed from the finding of vaginal plugs. The mean gestational period was found to be 21 days. Groups of mice were studied at 15–16, 17–19, and 20–21 days of gestation. FeL pregnant mice (FeLP) were given Imferon 2 wk before mating. The bioavailability of the iron injected in this manner was confirmed by the measurement of hemoglobin levels. In pregnant iron-loaded mice, HB was 14.0 ± 0.2 g/100 ml (mean ± SE) compared to 13.5 ± 0.3 in controls and 11.8 ± 0.4/100 ml in day 20–21 pregnant animals, indicating that iron loading prevented the development of anemia during the third week of pregnancy.

Hysterectomy was performed at days 20–21 of gestation. Under ether anesthetic, the abdomen was opened and the uterus mobilized. The blood supply was ligated, leaving the ovarian supply intact, and the uterus was divided below the cervix. The uterus and contents were removed in toto. The abdominal wall was closed in layers with black silk sutures and Michelle clips. A sham-operated group was prepared by opening the abdomen, mobilizing the uterus, and then replacing the structures within the abdominal cavity. Surgery was performed 16 h prior to study.

Analytical methods. Iron stores were assessed by measuring the hepatic nonheme iron content. It has been established that there is a close correlation between total body and hepatic nonheme iron levels (14). Nonheme iron levels were chemically determined by the method of Weinfeld (15).

The iron content of the diets was determined by sub-
4 h. The resulting ash was treated 3 times with 20 ml 3 N HCl and heated to 78°C in a water bath. The mixture was filtered through acid-washed Whatman 541 filter paper and the filtrate made up to 50 ml in a volumetric flask. Iron determinations were made using an atomic absorption spectrophotometer.

All glassware used was rendered iron free by soaking overnight in 6 N HCl and then rinsing in glass distilled, deionized water.

In vivo studies. Intestinal uptake (U) and transport (T) were determined 4 h after feeding a 1-µg dose of iron as ferrous ammonium sulfate labeled with 1 µCi 59FeCl₂ (Radiochemical Center, Amersham, United Kingdom).

Iron uptake (U) was defined as the amount of the dose leaving the intestinal lumen. The value was calculated by subtracting the radioactivity recovered in the intestinal washings, stomach, and colon from the dose administered and by expressing the result as a percentage of the administered dose. It was demonstrated in early experiments that the 59Fe content of the stomach and colonic wall was less than 0.1% of the dose administered. Thus, these were counted intact and the 59Fe present was assumed to represent unabsorbed iron. This calculation may overestimate iron uptake because it does not take into consideration the unknown quantity of 59Fe that remains within the unstirred water layer at the surface of the intestinal mucosa (12). T, a measure of the iron transferred from the small intestine to the surface of the small intestine (12). T, a measure of the percentage of the dose transferred from the intestine to the circulation, was determined directly by counting the radioactivity present in the carcass after removal of the entire gastrointestinal tract. T was expressed as a percentage of the dose administered.

Four hours after the administration of the dose in 0.2 ml via a polyethylene tube passed into the stomach under light ether anesthetic, the animals were killed. The stomach, small intestine, small and large bowel, liver, and spleen were separated from the carcass. The small intestine was immediately rinsed twice with 10- to 15-ml vol of normal saline at 4°C to remove any unabsorbed iron from the lumen. Mucus adherent to the surface of the intestinal mucosa was expressed by gentle compression. The entire gastrointestinal tract, liver, spleen, carcass, and, in the case of pregnant animals, uterus, placentas, and fetuses were counted in a Packard Auto-Gamma Spectrometer.

Division of the small intestine into duodenum, jejunum and ileum, revealed that the duodenum and proximal jejunum were the major sites of iron uptake in pregnant and nonpregnant mice.

Recovery in these experiments ranged from 88–102%, means 94.9 ± 0.6%. Maternal hepatic uptake of 59Fe is expressed as ng Fe²⁺. Fetal uptake is expressed as a percentage of the dose transported from the intestine. Placental radioactivity at 4 h is negligible, and thus 59Fe in the fetoplacental units represents in effect, fetal activity.

Studies of hepatic uptake were performed in control, pregnant, FeD, and hysterectomized mice following an intravenous dose of 59Fe calculated to avoid saturation of the total iron-binding capacity of the serum. The dose of 2 µg 59Fe in 0.2 ml as FeCl₂ was injected into the inferior vena cava under light ether anesthetic, and 5 min after injection, the animal was exsanguinated by cardiac aspiration. The liver was removed, washed in ice-cold saline, and flushed via the portal vein remnant with 15 ml of saline. Gel electrophoresis of mouse serum 5 min after injection of the 59Fe revealed that 85% of the iron remaining was bound to transferrin.

Statistical methods. The results were analyzed by the t test for unpaired groups. All values are shown as means ± SE. P values of < 0.05 have been taken as significant.

RESULTS

Effect of pregnancy on intestinal uptake and transport. 59Fe, U and T from the small intestine in control, pregnant, and FeD groups are shown in Fig. 1. A progressive increase in both U and T was demonstrated after days 15–16 of pregnancy. Maximum values for U, 29.2 ± 4.5% and T, 24.3 ± 3.8%, were reached on days 20–21. Each of these values in the late stages of gestation was significantly higher than corresponding values of 16.4 ± 2.3 and 10.7 ± 1.2% in a control group of nonpregnant mice, (P < 0.01).

U in late pregnancy was similar to that found in a group of FeD virgin females, 28.2 ± 1.0%. Although T increased from control levels of 10.7 ± 1.2 to 16.4 ± 4.5% in iron deficient mice (P < 0.05), the increase did not equal that found in late pregnancy, 24.3 ± 3.8%. Hepatic nonheme iron levels (Fig. 1) were reduced by 71% from control levels in FeD mice and by 77% in the days

![FIG. 1. Effect of pregnancy on intestinal 59Fe uptake and transport. Hepatic nonheme iron values are shown within histograms. Values are means ± SE. Results for iron deficient nonpregnant mice are included for comparison.](http://ajpendo.physiology.org/)
20-21 pregnant group. The levels were not statistically different in the two groups, and thus the higher T in pregnancy is unlikely to represent an effect of greater iron-store depletion.

Effect of parturition on uptake and transport. The initial indication that the increase in uptake and transport in pregnancy was not explicable on the grounds of a reduction in iron stores was provided by experiments in postpartum animals. Although hepatic iron-store depletion in this group was comparable to that in late pregnancy, uptake and transport fell 14-48 h after parturition. U at 48 h postpartum, 17.4 ± 1.3%, was similar to that in nonpregnant animals, 16.4 ± 2.7%. T, although less than that in pregnant animals, resembled that in the FeD group, 16.4 ± 4.5%.

Effect of iron loading on uptake and transport. Additional evidence that iron stores are not the sole determinant of the altered U and T was provided by experiments in pregnant iron-loaded mice (Fig. 3). Iron loading did not prevent the increase in intestinal uptake in pregnant animals. Values of 24.5 ± 7.2% were obtained in iron-loaded pregnant animals. This value was not significantly different from the level obtained in pregnant noniron-loaded mice, 29.2 ± 4.5%. Transport, 11.5 ± 3.7%, did not rise to the level found in days 20-21 mice, but was 3 times greater than the value obtained in iron-loaded nonpregnant animals.

Effect of hysterectomy on iron uptake and transport. The values for U and T in pregnant mice that had undergone hysterectomy at days 20-21 and in pregnant mice after a sham operation are shown in Table 1. Uptake decreased significantly within 16 h of the removal of the fetuses and placentas to 39.7 ± 4.9%, compared to 58.9 ± 9.6% in the sham-operated group (P < 0.05). Transport fell from 43.4 ± 9.6% to 25.7 ± 3.4% (P < 0.05) in the group undergoing hysterectomy. These experiments also show an increase in the percentage of the transported dose taken up by the liver in hysterectomized mice, 31.3 ± 2.7%, compared to sham-operated mice, 9.3 ± 1.5% (P < 0.01). Hepatic nonheme iron values were similar in the two groups. The value for hysterectomized mice was 44.6 ± 2.2 μg/g and for the sham-operated group, 37.9 ± 2.8 μg/g.

Effect of pregnancy on hepatic and fetal uptake after an oral dose of 59Fe. Whereas the quantity of an oral dose of iron transported by the small intestine doubled in pregnancy, hepatic uptake, 14.6 ± 3.0 ng, was less than in a control, 35.6 ± 7.2 ng (P < 0.01). Uptake in iron deficient, 89.5 ± 40.6 ng, and 48-h postpartum groups, 69.3 ± 14.2 ng, was significantly higher than in either pregnant or control animals (P < 0.01) (Table 2). When the values for hepatic uptake were expressed as a percentage of the radioactivity leaving the intestine, the lowest values for hepatic uptake, 6.7 ± 0.9%, were found in the pregnant group (Table 2). The corresponding value for fetal uptake was 75.5 ± 4.9%. The transport of this large fraction of dietary iron to the fetoplacental units was not affected by prior loading of the maternal iron stores. In experiments in a group of six iron-loaded pregnant mice, 85.8 ± 5.0% of the iron transported from the intestine was recovered in the fetuses.

Effect of pregnancy on hepatic uptake of an intravenous dose of 59Fe. Hepatic uptake 5 min after injection of 2 μg 59Fe is shown in Table 3. Uptake in pregnant mice, 9.7 ± 0.8%, was significantly lower than in controls, 13.1 ± 0.9% (P < 0.05). Within 5 min of hysterectomy, the liver of pregnant animals responded in a manner appropriate to the degree of iron depletion evident in late pregnancy. Uptake in the hysterectomized group rose to 18.6 ± 4.4% (P < 0.05).

**TABLE 1. Effect of hysterectomy on intestinal uptake and transport and hepatic uptake of an oral dose of iron in pregnancy**

<table>
<thead>
<tr>
<th>Group</th>
<th>Uptake, % Dose</th>
<th>Transport, % Dose</th>
<th>Hepatic Iron Uptake, % Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (5)</td>
<td>58.9 ± 9.6</td>
<td>43.4 ± 9.6</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td>Hysterectomized (8)</td>
<td>39.7 ± 4.9*</td>
<td>25.7 ± 3.4*</td>
<td>31.3 ± 2.7*</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Numbers in parentheses indicate group size. *P < 0.05 compared with sham-operated group. †P < 0.01 compared with sham-operated group.
TABLE 2. Effect of pregnancy on hepatic ⁵⁹Fe uptake from a 1-µg oral dose of iron

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic Uptake, µg</th>
<th>Hepatic Uptake, % Transformed Dose</th>
<th>Fetal Uptake, % Transformed Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (18)</td>
<td>35.6 ± 7.2</td>
<td>24.5 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Days 20-21 pregnant (13)</td>
<td>14.6 ± 3.0*</td>
<td>6.7 ± 0.9*</td>
<td>75.3 ± 4.9</td>
</tr>
<tr>
<td>FeD (6)</td>
<td>89.5 ± 40.6*</td>
<td>38.4 ± 11.5*</td>
<td></td>
</tr>
<tr>
<td>Postpartum (8)</td>
<td>69.3 ± 14.2*</td>
<td>40.2 ± 4.9*</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the means ± SE. Numbers in parentheses indicate group size. *P < 0.05 compared with control values.

TABLE 3. Effect of pregnancy and hysterectomy on hepatic ⁵⁹Fe uptake after an intravenous dose of iron

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic Uptake % Dose</th>
<th>Hepatic Nonheme Iron, µg/g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (7)</td>
<td>13.1 ± 0.9</td>
<td>198.1 ± 18.7</td>
</tr>
<tr>
<td>Days 20-21 pregnant (6)</td>
<td>9.7 ± 0.8*</td>
<td>99.6 ± 9.5f</td>
</tr>
<tr>
<td>Iron-deficient (6)</td>
<td>18.8 ± 2.1*</td>
<td>108.7 ± 11.7*</td>
</tr>
<tr>
<td>Hysterectomized (5)</td>
<td>18.6 ± 4.4*</td>
<td>37.8 ± 5.5*</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Numbers in parentheses indicate group size. *P < 0.05 compared with control values. tP < 0.01 compared with control values.

DISCUSSION

These experiments confirm the importance of iron stores in regulating iron absorption in the nonpregnant animal. Comparison with pregnant animals in which iron stores were reduced by fetal demands and in pregnant animals loaded with iron in the early stages of gestation revealed important differences in the control of iron absorption.

An increase in iron uptake from the small intestine was detected as early as day 16 of pregnancy, but it was greatest immediately prior to delivery. Levels at this time were similar to those found in nonpregnant, iron-deficient animals. Although depleted iron stores could thus account for the findings in pregnant mice, it is notable that iron uptake fell 12-48 h after delivery. This observation provided the initial evidence that a factor other than reduced iron stores was contributing to iron absorption in pregnancy because measurement of hepatic nonheme iron showed that iron stores were comparable in pregnant and postpartum mice.

Further evidence for an effect of pregnancy unrelated to iron status was obtained in the experiments with iron-loaded animals. Iron loading reduced intestinal uptake in nonpregnant animals as demonstrated in previous studies (5, 7), whereas pregnancy in iron-loaded mice resulted in a significant increase in uptake during the third week.

The results of the experiments in hysterectomized mice show that the fetoplacental units play an important part in maintaining increased uptake of iron in pregnancy. There was a sharp reduction in intestinal uptake and transport within 16 h of operation. The rapidity of the response suggests that the fetoplacental units exert an influence that acts at the level of the absorptive epithelium. The time taken for the pregnant mice to respond to hysterectomy is similar to that observed after a stimulus to increased erythropoiesis, and it is possible that the mechanism involved is similar to that postulated by Weintraub et al. (18) in their study.

The increase in intestinal transport in pregnant animals was greater than that in iron-deficient virgins. A proportion of this increase may have been due to maternal iron depletion because transport in pregnant animals was partially suppressed by loading with iron. It was also found that transport in postpartum mice, although less than that in late pregnancy, was comparable to transport in iron-deficient nonpregnant animals. These observations can be reconciled with the studies of Murray (15), who examined the effects of iron supplementation on intestinal iron transport from an oral dose of iron. This study was performed on day 16 of pregnancy in rats in which the average gestation period was stated to be 23 days. We have shown in the mouse and in studies to be reported in the rat that iron transport is maximal 1-2 days before parturition. Accordingly, the demonstration that iron supplementation prevented the increase in transport in the rat may reflect the abolition of an effect of iron depletion. The effect of the fetoplacental units may be exerted at a later stage of the third week. An additional finding of these experiments was that of the preference of fetuses for dietary iron. In both pregnant and iron-loaded pregnant mice, more than 70% of the transported iron was transferred to the fetuses.

Awai and colleagues (2, 3) have produced evidence that the transfer of iron to the fetus in late pregnancy is due to the preferential removal by the placenta of one of the iron atoms on the two binding sites present on the transferrin molecule. These studies, which have now been challenged (10), help to explain the findings that after parturition in the mouse there was an increase in hepatic iron uptake. The experiments in hysterectomized animals show that the liver regained the ability to take up iron from the circulation within minutes. Any explanation of this observation must take into account the speed with which it occurred and the ability of the liver to respond in the manner of iron-deficient animals.

This study has shown that the presence of the fetoplacental units is an important factor acting independently of the status of maternal iron stores to maintain the increased intestinal uptake and transport of iron in the pregnant animal. Fetuses and placentas also play an important part in determining the fate of iron entering the circulation from the intestine. Surgical removal of the fetoplacental units has been shown to abolish the increase in iron absorption and to increase the uptake of iron by maternal tissues.

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REFERENCES