Influence of gestational age and fetal iron status on IRP activity and iron transporter protein expression in third-trimester human placenta

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Iron is an essential nutrient during early human development (26). Its therapeutic range is narrow; therefore, its transport must be tightly regulated to avoid deficiency or excess states. Iron deficiency and excess significantly affect red blood cell, brain, heart, and liver development and function (5, 8, 22, 30). Fetal and neonatal organs are arguably at higher risk than organs of older children for injury from iron deficiency because of their rapid growth rates and organ development and from iron excess because of their low iron-binding capability (9) and poor antioxidant defenses (7). Perinatal iron deficiency occurs commonly in gestations complicated by maternal iron deficiency, intrauterine growth retardation, and maternal diabetes mellitus (10, 19, 20, 37), whereas iron overload occurs predominantly in the setting of congenital hemochromatosis (11).

Iron delivery to the fetus likely involves regulation of the placental expression of the proteins known to mediate vectorial iron transport across single-cell barriers, such as the duodenal epithelium, mammary gland, and blood-brain barrier. Because the syncytiotrophoblast takes up ferric iron bound to transferrin at the apical membrane, the likely transporter of interest at that site is transferrin receptor-1 (TIR-1) (6, 21, 38, 43). At the basal membrane, where iron is transported to the fetus, the putative transporters are ferroportin (FPN)-1 (13) and TIR-1 (43), both of which localize to this membrane at full term. These transporters, in turn, are postulated to be posttranscriptionally regulated by iron regulatory proteins (IRPs), which bind iron-responsive elements (IREs) found in the untranslated regions of the respective mRNAs (14). Although the responsiveness of the IREs on TIR mRNA is unquestioned, it remains unclear whether the IREs found on FPN-1 mRNA are active (31). Furthermore, which IRP is responsible for mRNA stabilization remains unclear (14). Genetic knockout of IRP-2 results in a mouse with significant iron abnormalities (27), whereas the IRP-1 knockout mouse has yet to be characterized.

TIR-1 and FPN-1 expression and IRP-1 RNA-binding activity have been demonstrated in full-term placenta (6, 13, 21, 38, 43). A preliminary study failed to demonstrate IRP-2 activity at full term (18). TIR mRNA and protein expression, as well as IRP-1 RNA-binding activity, appears to be responsive to fetal iron deficiency when maternal iron status is normal (18, 38). However, the normal developmental trajectory of the expression and regulation of these proteins throughout the third trimester of human pregnancies has not been studied. The specific objectives of this study were 1) to determine the developmental trajectory of TIR-1 and FPN-1 concentration and localization in human placenta during the third trimester and 2) to assess how third-trimester placental IRP-1 and IRP-2 activities relate to gestational age, calculated rate of placental iron transport, fetal/placental iron status, and transport protein expression.

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IRP-1 or IRP-2 band intensity was quantified using ImageQuant software. Three separate experiments were performed using fresh extracts.

**Western blot analysis.** For IRP-1 and IRP-2, total protein (50 μg) of placental extracts was separated by an 8% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were probed with chicken anti-rat IRP-1 antibodies or rabbit anti-rat IRP-2 antibodies (23). Membranes were probed with horseradish peroxidase-conjugated goat anti-chicken or goat anti-rabbit secondary antibodies for IRP-1 or IRP-2, respectively, and developed using Western Lighting Chemiluminescence Reagent Plus (Perkin-Elmer, Boston, MA).

For ferritin, Tir-1, and FPN-1, frozen placenta was pulverized in liquid nitrogen to a fine powder and immediately resuspended in protein lysis buffer (25 mM HEPES, pH 7.5, 40 mM KCl, 5% glycerol, 1 mM DTT, 0.5% NP-40, and 1 mM PMSF). The extracts were subsequently centrifuged for 10 min at 13,000 g at 4°C, and the supernatants were assayed for total protein concentration by the protein assay method (Bio-Rad, Richmond, CA). A total of 50 μg of placental protein was loaded per lane and electrophoretically separated on a 7.5% SDS-polyacrylamide gel. Protein was then transferred onto enhanced chemiluminescence (ECL) nitrocellulose membranes via semidry electrophoresis using a Tris-glycine transfer buffer. Membranes were probed for ferritin using a rabbit anti-human ferritin (H-type) antibody (Boehringer Mannheim, Indianapolis, IN); a horse-radish peroxidase-conjugated secondary antibody was used. Tir-1 was detected using an anti-Tir-1 primary antibody (Chemicon International, Temecula, CA) at a 1:100 dilution, and FPN was detected using a primary antibody (Alpha Diagnostics, San Antonio, TX) at a 1:500 dilution. A 1:3,000 dilution of ECL donkey anti-rabbit secondary antibody was applied to each, and protein visualization was accomplished using the ECL Western blotting analysis system (Amersham Pharmacia, Buckingham, UK). The relative optical density of the protein was standardized as a function of actin expression.

**Immunohistochemistry.** Serial 12-μm sections were obtained using a cryostat (model CM1900, Leica Instruments, Nussloch, Germany) at −25 to −27°C, mounted on poly-l-lysine-coated slides, and stored at −80°C until immunohistochemical analysis for Tir-1 was performed as previously described (38). The same primary mouse anti-human Tir-1 antibody (Chemicon) used in the Western blot analysis was used at a 1:100 dilution. For FPN, a rabbit antibody against human FPN-1 was generated against amino acids 223–230 fused to glutathione S-transferase. The FPN-1 antibody was used at a dilution of 1:400. For FPN-1, a preimmune serum control was also utilized and demonstrated no reactivity. In experiments for all three proteins, the primary antibody was omitted from the control slides, which did not demonstrate background staining.

After incubation, slides were treated for 30 min in a biotinylated secondary antibody to match the species of the primary antibody and then with streptavidin-peroxidase complex (Vector Laboratory, Burlingame, CA). The bound peroxidase was revealed by incubation of the time of delivery and frozen at −80°C until assayed. Six 1-in. 3 samples were obtained from the center of each cotyledon. Cord serum samples were collected at the time of delivery and frozen at −80°C until assayed for ferritin and erythropoietin concentrations.

**IRP-1 and IRP-2 RNA-binding activity assays.** IRP-1 and IRP-2 activities on placental specimens were assessed by RNA band shift assay as previously described (18). Approximately 100 μg of tissue were excised from each placental sample and homogenized in 3× weight (0.1 mg = 0.1 ml) lysis buffer (10 mM HEPES, pH 7.6, 10 mM KCl, 0.25% NP-40, 1.5 mM MgCl2, 0.5 mM DTT, and 1 mM EDTA) containing 1 mM PMSF and 1 mM dithiothreitol. The extracts were centrifuged for 20 min at 13,000 g at 4°C. Supernatants were assayed for total protein concentration using the Coomassie Plus protein assay reagent (Pierce Chemical, Rockford, IL). On the day of homogenization, 12 μg of total protein in 20 μl of binding buffer (10 mM HEPES, pH 7.6, 40 mM KCl, 5% glycerol, and 3 mM MgCl2) were incubated with an excess of 32P-labeled rat ferritin L-IRE RNA and subsequently treated with RNase T1 (20 U) and heparin (75 μg) (28). Samples were then incubated with rabbit anti-rat IRP-2 antibodies (23) for 20 min to supershift IRP-2 RNA complexes. Samples were analyzed by a 5% nondenaturing polyacrylamide gel and exposed to film or phosphor imaging for analysis.

**METHODS**

**Study population.** The study was approved by the Institutional Review Board of the University of Minnesota. Informed consent was obtained from all participating subjects. The research subjects were pregnant women delivering at United Hospital (St. Paul, MN). Twenty-one placental samples designed to cover all gestational age groups were collected from consecutively consenting subjects. The recruitment was unbiased with respect to maternal age, parity, and race. Gestational age was determined by maternal dates when reliable, first- or second-trimester ultrasound when dates were unreliable, and physical examination for gestational age when the first two indicators were not present. Infants were weighed and measured, and the data were plotted on a standard growth curve to determine appropriateness of intrauterine growth and to rule out intrauterine growth retardation (4). Infants with birth weight z scores less than −1.5 or greater than +1.5 were excluded. Maternal charts were screened for the presence of confounding factors for fetal iron status. Pregnancies complicated by medical conditions that have been shown to affect fetal iron status, including maternal iron deficiency, small-for-gestational age infant at birth, maternal hypertension with intrauterine growth retardation, maternal diabetes mellitus, and neonatal infection, were excluded (10, 16). Seventy-one percent of mothers took prenatal iron supplements, and none had indexes of iron deficiency at delivery. Fifteen mothers delivered before 37 wk of gestation. The causes for premature deliveries are presented in Table 1.

**Placental tissue and serum collection.** The collection was targeted to provide a minimum of four placentas at five gestational ages: 24–27, 28–30, 31–33, 34–37, and 38–40 wk. The placentas were obtained within 5 min of delivery via RNase-free technique and stored at −80°C until assayed. Six 1-in. 3 samples were obtained from the center of each cotyledon. Cord serum samples were collected at the time of delivery and frozen at −80°C until assayed for ferritin and erythropoietin concentrations.

**Table 1. Clinical characteristics of the study population**

<table>
<thead>
<tr>
<th>EGA Group</th>
<th>24–27 wk (n = 4)</th>
<th>28–30 wk (n = 4)</th>
<th>31–33 wk (n = 4)</th>
<th>34–37 wk (n = 5)</th>
<th>38–40 wk (n = 5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGA, wk</td>
<td>25.8 ± 1.3</td>
<td>29.0 ± 0.8</td>
<td>32.8 ± 0.5</td>
<td>35.3 ± 1.5</td>
<td>39.4 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body wt z score</td>
<td>−0.7 ± 1.3</td>
<td>0.4 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td>0.3 ± 0.6</td>
<td>0.6 ± 0.9</td>
<td>0.64</td>
</tr>
<tr>
<td>Maternal age, yr</td>
<td>24 ± 4</td>
<td>29 ± 10</td>
<td>26 ± 7</td>
<td>34 ± 6</td>
<td>28 ± 6</td>
<td>0.32</td>
</tr>
<tr>
<td>Maternal Hb, g/l</td>
<td>112 ± 13</td>
<td>105 ± 14</td>
<td>115 ± 12</td>
<td>116 ± 11</td>
<td>109 ± 15</td>
<td>0.76</td>
</tr>
<tr>
<td>In (cord EPO), U/l</td>
<td>3.1 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>2.3 ± 1.4</td>
<td>2.9 ± 0.1</td>
<td>3.6 ± 0.4</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Values are means ± SD. EGA, estimated gestational age; EPO, erythropoietin. There were 16 premature deliveries at <37 wk. Causes of prematurity included multiple births (7), premature rupture of membranes (7), oligohydramnios (2), cervical incompetence (1), and pregnancy-induced hypertension without intrauterine growth retardation (1). Two pregnancies had both twins and premature rupture of membranes. *P value was based on F value for 1-way ANOVA.
the sections in 3,3′-diaminobenzidine (Vector Laboratory). Slides were visualized under a light microscope through a ×100 objective (Eclipse E600, Nikon, Melville, NY) and digitized to a computer by a Nikon DMI1200 digital charge-coupled device camera. The images were stored on a Dell Dimension 8200 computer in Adobe Photoshop (version 6) and assessed using ACT-1 software (version 2.20, Nikon).

TIR-1 and FPN-1 protein localization was assessed in each gestational age group by visual inspection. TIR-1 was assessed on the apical and basal trophoblastic membranes, and FPN-1 was assessed on the basal membrane in the location previously described by Donovan et al. (13).

Biochemical assays. Cord serum ferritin concentrations were determined in duplicate by chemiluminescent immunoassay (Access Immunoassay System, Beckman-Coulter, Brea, CA) as previously described (18). Cord serum erythropoietin concentrations were graciously assayed by Dr. John Widness as previously described (19). Placental nonheme iron concentration was determined by atomic absorptiometry after digestion with nitric-perchloric acid as previously described (18). Cord serum erythropoietin concentrations were graphically assessed by comparing the IRP activities and transporter/storage protein expressions of the placentas from the highest tertile of cord serum ferritin with those from the lowest tertile. Values are means ± SE. Statistical significance was set at \( P < 0.05 \).

RESULTS

Table 1 demonstrates the gestational age group characteristics of the sample and the reasons for premature birth. Birth weight \( z \) scores, maternal hemoglobin concentration, and cord serum ferritin concentrations were not different among the groups. Cord erythropoietin concentrations were low \( (19) \) and did not correlate with gestational age \( (r = 0.16) \), suggesting that none of the fetuses were hypoxic and that the infants delivered prematurely were not more hypoxic than the full-term infants. In addition, we found no significant relation between maternal hemoglobin and cord hemoglobin \( (r = 0.005) \) or natural logarithm of erythropoietin \( (r = 0.33) \) or ferritin concentrations \( (r = -0.28) \).

Gestational age group did not have a significant effect on serum ferritin concentrations (Table 2, Fig. 1A). These findings are consistent with previously published data \( (40) \) and with the working model that the fetus maintains a relatively stable amount of storage iron per kilogram of body weight throughout the third trimester \( (36) \). In contrast, placental ferritin protein expression was highly positively correlated with gestational age \( (Fig. 1B) \), implying that the placenta stores a large amount of iron toward the end of gestation, potentially serving as an iron reservoir for the fetus. Serum ferritin concentration and placental ferritin protein expression were correlated \( (r = 0.59, P < 0.01) \). Cord serum ferritin concentration and placental ferritin protein expression were not positively related to maternal hemoglobin concentration.

IRP-1 and IRP-2 activity was present throughout the third trimester, in contrast to our previous study where IRP-2 activity was not detected in full-term placentas \( (18) \) (Fig. 2). IRP-1 activity remained constant until the end of gestation, at which point it decreased significantly (Table 2). IRP-2 activity did not change with gestational age. The IRP-1 activities of placentas from the lower tertile of cord serum ferritins were significantly higher than those from the upper tertile (Table 3). Furthermore, the cord serum ferritin concentration correlated inversely with IRP-1 \( (Fig. 3A) \) and IRP-2 \( (Fig. 3B) \) activities, suggesting that both proteins respond to the fetal iron status. Placental ferritin protein expression correlated better with IRP-1 \( (r = -0.46, P = 0.04) \) than with IRP-2 \( (r = -0.35, P = 0.10) \) activity.

Table 2. Fetal and placental iron parameters

<table>
<thead>
<tr>
<th>EGA Group</th>
<th>24–27 wk</th>
<th>28–30 wk</th>
<th>31–33 wk</th>
<th>34–37 wk</th>
<th>38–40 wk</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated iron transport, mg/kg ( \cdot ) wk (^{-1} )</td>
<td>120 (4)</td>
<td>80 (4)</td>
<td>80 (4)</td>
<td>109 (5)</td>
<td>115 (5)</td>
<td>NA</td>
</tr>
<tr>
<td>Cord serum ferritin, ( \mu )g/l</td>
<td>95±48</td>
<td>86±48</td>
<td>88±30</td>
<td>74±18</td>
<td>150±68</td>
<td>0.17</td>
</tr>
<tr>
<td>PNHI, ( \mu )g/dry wt</td>
<td>202±75</td>
<td>201±36</td>
<td>191±49</td>
<td>247±47</td>
<td>233±100</td>
<td>0.56</td>
</tr>
<tr>
<td>Placental IRP-1 activity, OD</td>
<td>3.4±0.86</td>
<td>2.7±0.91</td>
<td>3.4±0.89</td>
<td>3.8±0.93</td>
<td>1.3±0.71</td>
<td>0.007</td>
</tr>
<tr>
<td>Placental IRP-2 activity, OD</td>
<td>0.51±0.43</td>
<td>0.51±0.16</td>
<td>0.68±0.43</td>
<td>0.61±0.33</td>
<td>0.28±0.07</td>
<td>0.38</td>
</tr>
<tr>
<td>Placental TIR-1, OD</td>
<td>2.8±1.5</td>
<td>3.0±0.8</td>
<td>3.0±1.6</td>
<td>2.7±1.1</td>
<td>3.0±1.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Placental FPN-1, OD</td>
<td>1.7±0.6</td>
<td>1.8±0.6</td>
<td>1.3±0.4</td>
<td>1.6±0.6</td>
<td>3.0±1.5</td>
<td>1.01</td>
</tr>
<tr>
<td>Placental ferritin, OD</td>
<td>4.1±3.0</td>
<td>2.4±1.7</td>
<td>3.4±1.2</td>
<td>8.8±2.8</td>
<td>13.2±3.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD. PNHI, paroxysmal nocturnal hemoglobinuria; IRP, iron regulatory protein; TIR, transferrin receptor; FPN, ferroportin; OD, optical density units; NA, not applicable. \( P \) value was based on \( F \) value for 1-way ANOVA. *Western blot.
TfR-1 and FPN-1 were detectable at 24 wk of gestation and were present throughout the third trimester. The primary location of the transport proteins did not change with gestational age. TfR-1 was found predominantly on the apical trophoblastic membrane but was also present on the basal membrane, consistent with previous reports (43). FPN-1 was localized exclusively to the basal membrane (Fig. 4). Regression analysis demonstrated that gestational age had no effect on the protein expression of TfR-1 (r = 0.05, P = not significant). FPN-1 expression increased with gestational age (r = 0.44, P < 0.05). Within the range of iron status tested, TfR and FPN-1 did not correlate significantly with cord serum ferritin (r = -0.29 for TfR and r = 0.20 for FPN-1) or IRP-1 or IRP-2 activity.

### DISCUSSION

The iron status of the newborn may in large part determine the risk of subsequent postnatal iron deficiency. Neonatal iron status is primarily a function of third-trimester maternal-placental-fetal iron transport. The present study provides novel information about the regulation of this transport. This study demonstrates that the major iron transporters, TfR-1 and FPN-1, as well as both mRNA-stabilizing IRPs, are present in the placenta throughout the third trimester. We were able to assess the effects of gestational age and fetal/placental iron status on iron transporter expression and regulation by studying an iron-sufficient maternal population that gave birth to infants with a range of fetal iron status. Gestational age had little effect on any of the measured parameters until near term, when IRP-1 activity decreased and placental ferritin and FPN-1 concentrations increased. The lack of change in cord serum ferritin concentrations, IRP activity, and transporter expression from 24 to 37 wk of gestation is consistent with a relatively constant delivery of iron on a per-weight basis to the fetus during this time period (36). The increases in placental ferritin and FPN-1 concentration at term suggest increased iron storage by the placenta and a modest increase in placental-fetal iron transport at the end of gestation. These findings are consistent with the large increase in total iron content of the fetus associated with fetal growth near term (36).

### Table 3. Comparison of placental iron transporters, iron storage proteins, and IRP activities from placentas with the lowest and highest tertiles of cord ferritin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Lowest Tertile (n = 7)</th>
<th>Highest Tertile (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord serum ferritin, µg/l</td>
<td>55±15</td>
<td>155±49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental IRP-1 activity, OD</td>
<td>3.29±0.84</td>
<td>2.18±1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Placental IRP-2 activity, OD</td>
<td>0.67±0.33</td>
<td>0.41±0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>Placental TfR-1,* OD</td>
<td>3.29±1.38</td>
<td>2.42±0.97</td>
<td>0.21</td>
</tr>
<tr>
<td>Placental FPN-1,* OD</td>
<td>1.89±0.53</td>
<td>2.19±1.50</td>
<td>0.64</td>
</tr>
<tr>
<td>Placental ferritin,* OD</td>
<td>3.68±3.39</td>
<td>8.93±5.77</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD. P value was based on F value for 1-way ANOVA. *Western blot.
This study also demonstrates that IRP-2 is present in the placenta throughout the third trimester. Both IRPs appear to respond to fetal iron status, with a tighter relation to IRP-1 than to IRP-2. The variance of IRP activity based on iron status suggests that the regulatory system is intact beginning at ≥24 wk of gestation. Two relevant iron transporters, TfR-1, divalent metal transporter-1 (DMT-1), and FPN-1, based on the form of iron presented to the apical surface of the single cell layer and on the mechanism of basal surface transport. When iron is presented to the apical surface from a serum source (e.g., at the blood-brain barrier or to the trophoblast), it is in its ferric form bound to transferrin. Diferric transferrin has a high affinity for TfR, which incorporates the complex into the cell via an endosome. Subsequent processing includes acidification of the endosome and transport across the endosomal membrane of the resultant ferrous iron by DMT-1 (29). Iron presented to the apical surface in its ferrous form is imported via DMT-1 located on the apical surface membrane. This mechanism appears to be unique to the duodenal epithelial cell, because it is the only surface that receives non-serum-derived iron (3).

The mechanism of vectorial iron transport across a basal cell membrane (usually into a serum compartment) is controversial. The intestine is the most studied model and is likely most analogous to the placenta. TfR-1 and FPN-1 are expressed on the basolateral membrane in the duodenal epithelial cell and provide alternative (or complementary) mechanisms for iron efflux (3, 32, 41, 44). In the intestine, FPN-1 is hypothesized to transport ferrous iron at this surface by providing a site for donation of a positive charge from a copper-containing protein to reconvert the iron to its ferric state. Ceruloplasmin and hephaestin are the leading candidates for this copper-mediated mechanism (2). The resultant ferric iron is hypothesized to bind to serum transferrin before shuffling into the vascular space. TfR-1 may also play a direct role in uptake of transferrin from the serum adjoining the basal surface and directly obtaining iron intracellularly (32).

In the present study, placental FPN-1 expression was localized to the basal membrane of the syncytiotrophoblast and increased significantly with gestational age. TfR-1 was localized to the apical and basal membranes and did not change with gestational age. The increase in FPN-1 expression is potentially consistent with increased placental-fetal iron transport, because the fetus grows and the blood volume expands proportionately as the third trimester progresses. Nevertheless, FPN-1, DMT-1, and TfR-1 are localized to the region of the basal membrane (13, 17, 21, 43), and others have demonstrated that DMT-1 and TfR-1 expression is also responsive to iron status (17, 43). Although DMT-1 staining of the basal membrane is discontinuous (21), we confirmed in this study that FPN-1 staining is continuous (13). Our immunohistochemical techniques are not sensitive enough to detect whether the basal structures expressing these two ferrous iron transporters are identical. We also visualized TfR-1 on the basal membrane, as has been previously reported (43). At this time, the data point to the possibility that any of the transporter systems expressed on the basal membrane of the syncytiotrophoblast may be the primary one (13, 17, 43) and that redundancies may exist to provide more precise regulation of fetal iron delivery.

![Graph A](image1.png)  
**Fig. 3.** IRP-1 (A) and IRP-2 (B) activity as a function of cord serum ferritin concentration. For IRP-1, \( r = -0.66, P < 0.001 \). For IRP-2, \( r = -0.42, P = 0.05 \).
The regulation of the protein expression of the iron transporters remains under investigation. The most thoroughly characterized system is the coordinate posttranscriptional regulation of TfR-1 and ferritin mRNA (14). During iron-deficient states, IRPs bind the IREs in the 3'-untranslated region of TfR and prevent binding of RNases. The consequent preservation of TfR-1 mRNA produces more copies of TfR-1. Simultaneously, IRPs also bind IREs in the 5'-untranslated region of ferritin mRNAs, preventing ribosomal binding of the 40S ribosomal subunit and subsequent translation. The result is reduction in available ferritin for intracellular iron storage. Coupled with an increase in cellular iron uptake via TfR-1, iron homeostasis is restored. The present study demonstrates that the IRP system is active throughout the third trimester and that IRP-2 is present in addition to IRP-1, in contrast to our previous report that found no detectable IRP-2 activity in full-term placenta (18). At that time, we speculated that IRP-2 was absent in the placenta or was present in levels too low to be detected by our methods. Given the larger sample size in this study, it appears that methodological issues may have prevented us from detecting IRP-2 in the previous study. Because placental extracts were assayed on the day of preparation in the present study, we conclude that IRP-2 may be particularly susceptible to degradation in frozen extracts.

As would be predicted, reduced levels of fetal and placental ferritin throughout the third trimester were associated with higher levels of IRP-1 in particular. In contrast, there was not sufficient variance of TfR-1 expression in these iron-sufficient placentas to allow us to reach any conclusions about TfR-1 regulation by the IRPs. On the basis of our previous observations in iron-deficient full-term placenta (18, 38), we had hypothesized a direct relation between placental TfR-1 and IRP-1 to increase placental importation of iron in response to decreased fetal stores. However, Western blot analysis showed that this relation (although trending in the right direction; Table 3) did not reach statistical significance. Similarly, FPN-1 concentrations did not change in relation to the IRPs or to iron status. As with TfR-1, this may also be due to lack of a wide enough range of iron status in this study or may support the hypothesis that the IREs found on FPN-1 mRNA are not functional. Much less is known about the regulation of FPN-1 than TfR-1. mRNA of FPN-1 contains IRE or IRE-like elements, suggesting that they may also be posttranscriptionally regulated. However, the functionality of FPN-1’s IREs is highly debated. Sequences within and flanking the IRE appear to contribute to differences in function of these RNA regulatory elements and may determine which IRP interacts with specific IRE-containing mRNA (42). Such factors include the distance of the IRE from the 5' end of the mRNA, the secondary structure of the IRE stem region, and the presence or absence of flanking sequences that stabilize the IRE structure (12, 24, 25). Interestingly, distance of the IRE from the 5' end of FPN-1 mRNA appears to vary in a specific manner, and this may contribute to the selective and divergent effects of iron on FPN-1 regulation (1).
Debate has centered on the individual roles of the two IRPs in mammalian biology (14). IRP-1 is an iron-sulfur containing constitutively expressed cytoplasmic protein that assumes greater IRE-binding activity in its apo form than in its saturated form (14). The apo form predominates during intracellular iron-deficient states. In contrast, the concentration of IRP-2 varies with iron status, increasing during periods of iron deficiency (18). Both have been shown to bind active IREs in the homoygous IRP-1 knockout mouse. Deletion of IRP-2 in a mouse leads to significant iron overload of the intestine and brain (27). Our study suggests that IRP-1 activity is more closely related to fetal and placental iron parameters than IRP-2. Interestingly, a recent report suggests that deletion of IRP-1 in the mouse interferes with early developmental stages and, therefore, may disrupt early embryonic survival (15). The finding suggests that IRP-1 may play an important role in establishment and maintenance of pregnancy.

In a human-based study population such as this, there are a number of potential confounders to consider. Our assessment of pregnancies throughout the third trimester necessitated the analysis of premature births, which are by definition abnormal events. Subjects with risk factors known for fetal iron abnormalities [which can also result in prematurity (39)], including diabetes mellitus, intrauterine growth retardation, and infection, were excluded. However, it is impossible to completely rule out the possibility that other factors leading to premature birth may have altered fetal iron status or placental iron transport regulation. Examples would include multiple gestations or fetal hypoxia. Seven of the preterm deliveries were secondary to multiple gestation (i.e., twins and triplets). The independent effect of multiple gestation on iron transport and regulation has not been reported. However, unpublished data from our 1987 study showed no differences in neonatal iron status indicators between twins and controls. Fetal hypoxia, which in turn could alter iron distribution (10), may result from a failing placenta before delivery of a premature infant. The generally low and similar cord serum erythropoietin concentrations in preterm deliveries compared with full-term deliveries may imply that prematurely delivered infants in this study were not more hypoxic as fetuses. The highest individual natural erythropoietin concentrations in preterm deliveries compared with full-term deliveries were generally low and similar cord serum erythropoietin concentrations. In summary, this study demonstrates the presence of a regulated iron transport system in the placenta during the third trimester in the human. The proteins that are likely to be involved in maternal-fetal iron transport localize to the same membrane surfaces at 24 wk and at full term. Furthermore, IRP-1 and IRP-2 activities are present throughout this part of gestation and respond in predictable manners to fetal and placental iron status. As it nears full term, the placenta stores large amounts of iron as ferritin under the direction of IRP-1, while the presence of increasing FPN-1 expression at the same time suggests an increase in placental-fetal iron transfer.

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REFERENCES


