

Distribution of brain iron, ferritin, and transferrin in the 28-day-old piglet

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Abstract:

This study sought to gain understanding of the effect of iron deficiency anemia on regional brain iron, ferritin, and transferrin concentrations in the growing piglet. We wanted to use an animal whose development is more similar to human development than rodent models. Fourteen 1-month-old piglets were used for this study. One group (n = 7) received supplemental iron (100 mg iron dextran injected intramuscularly) at postnatal day 2; the second group (n = 7) received no iron injection and became iron deficient anemic. Anemia was verified in the iron deficient group [hemoglobin of 41 g/L, compared with 112 g/L in controls and hematocrit (15%, compared with 34% in controls)] when piglets were 28 days old. Brain iron concentrations were significantly lower due to iron deficiency (P = 0.02). The H-chain to L-chain (H:L chain) ferritin ratio was decreased in response to iron deficiency with the exception of substantia nigra and deep cerebellar nuclei, which were significantly higher than controls (P < 0.05). Compared with adult levels, brain iron concentrations were much lower and transferrin much higher in the piglets, suggesting a very active iron uptake system even in control piglets. A much lower H:L chain ferritin ratio (P < 0.05) in adult brain regions indicates a shift in the ferritin isoform expression in brain either as a function of increasing iron concentration or development. These data indicate that the brain of the 28-day-old piglet is actively acquiring iron regardless of the administration of exogenous iron.

Keywords: brain; iron; ferritin; transferrin; pig; iron deficiency anemia

Article:

Introduction

This project explores the possibility of using the growing pig as an animal model for investigating changes in brain iron metabolism due to iron deficiency. Historically, the pig has been used as a model for researching cardiovascular and renal physiology as well as drug metabolism.¹⁻² The pig also has been utilized as a model for studying human nutrition (reviewed by Miller and Ullrey³). Because it is a monogastric mammal, the pig metabolizes nutrients including iron similarly to humans. Further, because mother's milk of both humans and pigs is very low in iron, the nursing piglet is an excellent model for iron deficiency anemia in nursing children.³

The piglet grows very rapidly (1000% increase in body weight during the first 6 weeks of life) compared with the human infant (200% increase in body weight during the first 6 weeks of life). This rapid growth, combined with the fact that newborn piglets have an extremely low body storage of iron at birth, leads to inevitable iron deficiency anemia in the suckling pig reared in farrowing pens with cement floors. Because the piglet has an iron requirement of approximately 7 mg daily to maintain hemoglobin' and iron in mother's milk is low (<1 ppm),³ without supplemental iron, the piglet's body stores will be depleted rapidly. Iron deficiency is not very prevalent in piglets reared noncommercially, because they can acquire iron from the soil.⁵

It is well established that iron dextran (100-200 mg) injected intramuscularly into piglets within several days of birth will prevent iron deficiency anemia.⁶⁻⁸ If piglets are weaned at 3 weeks of age, then 100 mg is adequate; otherwise, 150-200 mg is required.⁵ Once injected into the piglets, the iron dextran complex is presumed to be slowly removed from the injection site by transferrin and then incorporated into essential body iron or storage.⁹

Table 1 Hematological and liver non-heme iron data for one-month-old piglets

	Iron suppl. (CN)	Iron def. (ID)	P-value
Hematocrit	0.34 ± 0.0101	0.154 ± 0.015	<0.01
Hemoglobin (g/L)	112.1 ± 3.7	40.9 ± 0.59	<0.01
Serum Fe (μmol/L)	7.68 ± 1.6	3.96 ± 0.75	<0.05
Total iron binding capacity (μmol/L)	102.8 ± 4.4	147.2 ± 11.9	<0.01
Transferrin saturation (%)	7.8 ± 1.8	2.7 ± 0.6	<0.05
Liver iron (μmol/g tissue)	1.15 ± 0.2	0.46 ± 0.03	<0.01

Values are Means ± SEM for each parameter. P-values were determined by *t*-tests.

Given this high need for exogenous iron in early life, we wanted to investigate the sensitivity of the growing piglet brain to variations in iron supply by comparing iron dextran supplemented piglets (control) to unsupplemented piglets (iron deficient anemic). Relative to body size and growth rates, pig brain growth is more similar to humans than many other mammalian species.^{10,11} Thus, certain aspects of central nervous system development that may be iron dependent can be examined efficiently in pigs. One such aspect is myelination of the central nervous system.^{12,13} The piglet brain begins myelination in utero and peaks by 2 to 3 months postnatally.¹⁴ This process is similar to humans with respect to the in utero beginning of myelination and post-natal progression.¹¹ In contrast, the rat begins myelination on postnatal day 2 and peaks by postnatal day 14. Previous work from our laboratory¹⁵ and others¹⁶ demonstrate that brain regional distribution of iron, ferritin, and transferrin is affected by iron deficiency early in life. In addition, there are clearly documented changes in neurotransmitter metabolism and behavior in piglets.¹⁷⁻²¹

To gain a better understanding of how the developing pig brain metabolizes iron, this study examines the regional distribution of brain iron, ferritin, and transferrin at 1 month of age. Furthermore, by comparing the distribution of these parameters in the brains of iron dextran supplemented piglets to unsupplemented piglets, one can gain a suggestion as to which regions of the brain are more sensitive to systemic iron delivery.

Materials and methods

Animals

Fourteen (four female and ten male) 28-day-old Yorkshire piglets, obtained from sows within the Pennsylvania State University Animal Husbandry Facility, were used for this experiment. All methods were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Seven piglets received 100 mg iron dextran via intramuscular injection on postnatal day 2 (*n* = 7; five male, two female) (CN), and seven did not receive an iron injection (*n* = 7; five male and two female) (ID). The two groups (CN and ID) were housed in farrowing pens with cement floors and had free access to their mother's milk and water. On the morning of the experiment, animals were anesthetized with pentobarbital and the aorta was exposed. A 17 gauge butterfly needle was inserted, blood obtained, and the brain perfused with 1 to 2 L of ice cold phosphate buffered saline (pH 7.3).

Hematology and tissue non-heme iron

Hemoglobin (Hb) and hematocrit (Hct) were measured in blood samples acquired from the abdominal aorta. Hct was determined by centrifugation of blood collected in heparinized microcapillary tubes. Hb concentration was measured colorimetrically by the cyanmethemoglobin method using reagents purchased from Sigma Chemical Co. (Sigma Kit No. 525, Sigma Chemical Company, St. Louis, MO). Remaining blood was centrifuged at 3000 rpm for 15 minutes at 4°C, and plasma was removed and frozen at -20°C.

The liver was removed and frozen at -70°C until it was analyzed. Liver non-heme iron was measured in a 1 g section following acid hydrolysis using a spectrophotometric technique according to Torrance and Bothwell.²² Ferrozine was the color reagent used. Plasma iron concentration and total iron binding capacity were measured using a Ferrochem II analyzer (ESA, Inc., Bedford, MA).

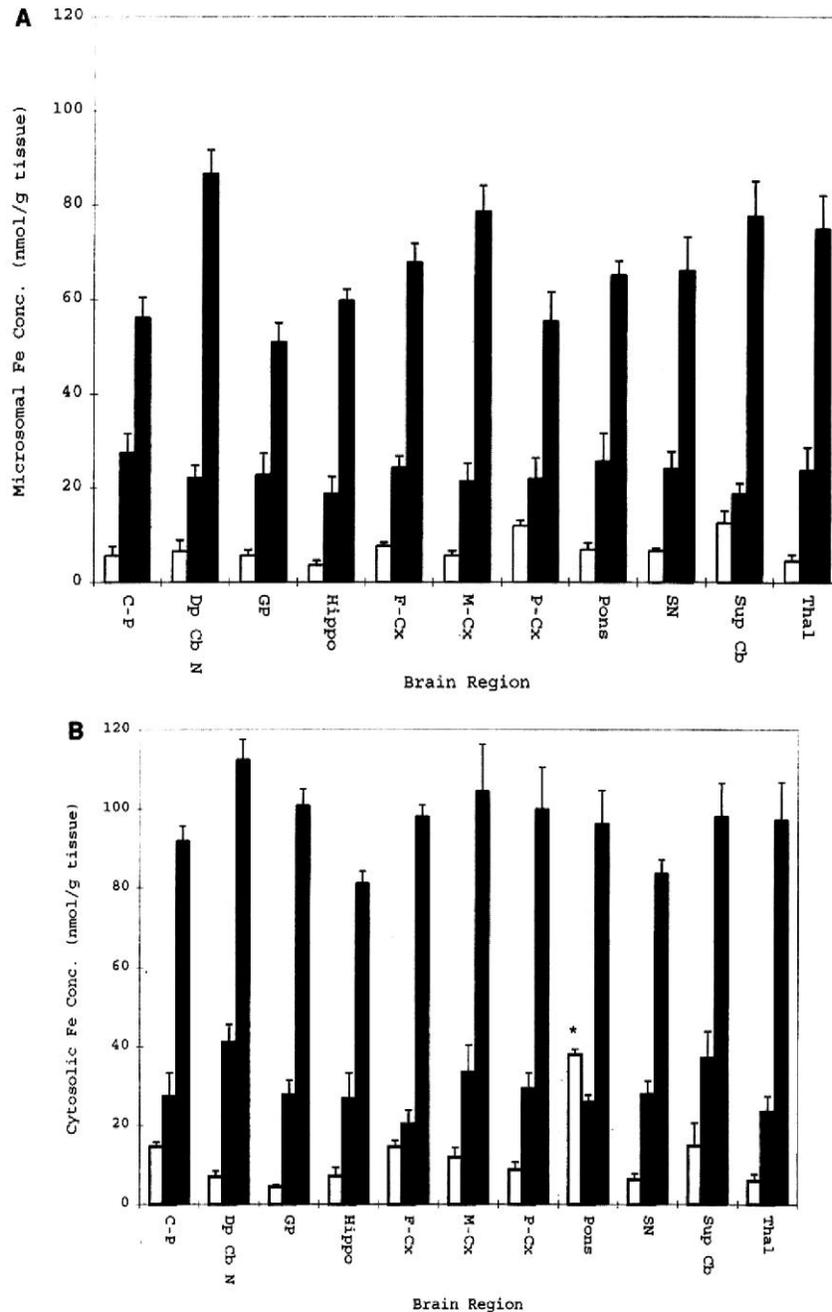


Figure 1 Regional brain iron concentrations for iron deficient piglets (ID) ($n = 5$), control piglets (CN) ($n = 5$), and an adult pig (AD) (replicates = 6) determined in microsomal (Figure 1A) and cytosolic (Figure 1B) fractions. Brain regions examined were caudate putamen (CP), deep cerebellar nuclei (Dp Cb N), globus pallidus (GP), hippocampus (Hippo), frontal cortex (F-Cx), medial cortex (M-Cx), posterior cortex (P-Cx), pons, substantia nigra (SN), superficial cerebellum (Sup Cb), and thalamus (Thal). (Figure 1A) Iron deficiency had an overall main effect of lowering microsomal regional brain iron ($P < 0.05$). Adult regional brain iron concentrations in microsomal fractions were significantly higher than in piglets ($P < 0.01$). Standard error bars represent replicate variation in the adult pig (AD) and animal variation in the piglets (ID) and (CN). (□ = ID Cyt; ■ = CN Cyt; ▨ = AD Cyt) (Figure 1B) Iron deficiency had an overall main effect of lowering cytosolic regional brain iron ($P < 0.05$), except for pons indicated by (*). Adult regional brain iron concentrations in cytosolic fractions were significantly higher than CN ($P < 0.01$). Standard error bars represent replicate variation in the adult pig (AD) and animal variation in the piglets (ID) and (CN). (□ = ID Micro; ■ = CN Micro; ▨ = AD Micro)

Microsomal and cytosolic brain iron

Brains were rapidly removed from animals and immediately dissected on cold dissection blocks into ten brain regions: frontal cortex (F-Cx), medial cortex (M-Cx), posterior cortex (P-Cx), hippocampus (Hippo), pons, caudate putamen (CP), globus pallidus (GP), substantia nigra (SN), superficial cerebellum (Sup Cb), deep cerebellar nuclei (Dp Cb N), and thalamus (Thal). The dissected regions were immediately frozen at -70°C . The brain regions were later homogenized 1:5 (w/v) in ice cold buffer H, which contained 20 mmol/L HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid), 250 mmole/L sucrose, 1 mmol/L EDTA (ethylenediaminetetraacetic acid), 100 $\mu\text{mol/L}$ leupeptin, and 100 $\mu\text{mol/L}$ PMSF (phenylmethylsulfonyl fluoride), at pH 7.2 using an Ultra-Turrax homogenizer (Tekmar Co. Cincinnati, OH) at high output for 30 seconds. All steps

were carried out at 4°C. The homogenates were centrifuged at 1000 X g for 10 minutes and the crude supernatant was collected and centrifuged at 21,000 x g for 20 minutes (Beckman L-8 70 ultracentrifuge with Beckman 70 Ti rotor). The resulting supernatant was subjected to centrifugation at 100,000 X g for 60 minutes (same centrifuge and rotor as previous centrifugation), yielding a microsomal pellet and cytosolic supernatant. Aliquots of these fractions were diluted with 0.2% ultra pure nitric acid and analyzed for iron by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 5100 AA) (adapted from Chen et al.²³). Liver fractions and adult pig brain tissue that was acquired from the Pennsylvania State University Meat Laboratory were analyzed in an identical manner. The liver fractions were analyzed for inter-organ comparison, whereas the adult pig brain tissue were analyzed for developmental comparison to the young pig brain tissue.

Transferrin and ferritin immunoblot

The immunoblot assay was performed according to the slot blot technique.¹⁶ The concentration of the protein for each sample loaded into slots was 5 µg/100 µl. Micro-Lowry protein determination was utilized to ensure that proper protein concentration was loaded (Sigma procedure #P5656, Sigma Chemical Co.). The primary antibodies were used at a 1:2000 dilution and the secondary antibodies were diluted 1:5000. The ferritin primary antibodies were murine monoclonal antihuman H-chain ferritin and antihuman L-chain ferritin (Ramco Laboratories, Houston, TX); the transferrin primary antibody was goat antipig (Bethyl Labs, Montgomery, TX). The secondary antibody, used against the ferritin primary antibody was antimouse IgG alkaline phosphatase conjugated, and the secondary antibody used against the transferrin primary antibody was antigoat IgG alkaline phosphatase conjugate (Sigma Chemical Co., catalog numbers A-5324 and A-4062, respectively). After secondary antibody treatment the blots were developed using the NBT/BCIP (nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) color substrate system. An internal standard comprised of a pool of liver cytosolic fractions was run on every membrane to ensure quality control between assays. All measurements were performed with standardized conditions using an Eagle-Eye densitometer (Stratagene, San Diego, CA).

Statistical analysis

A t-test for samples with unequal variance was used to analyze hematologic and liver iron data. Correlational analysis, one-way analysis of variance (ANOVA) for unequal variance and Tukey's post-hoc analyses (when main effects were significant) were used to analyze brain iron, ferritin, and transferrin data.²⁴ Box plot analysis was used for identifying outliers, which were then excluded (Minitab, State College, PA). A p-value of less than 0.05 was considered significant.

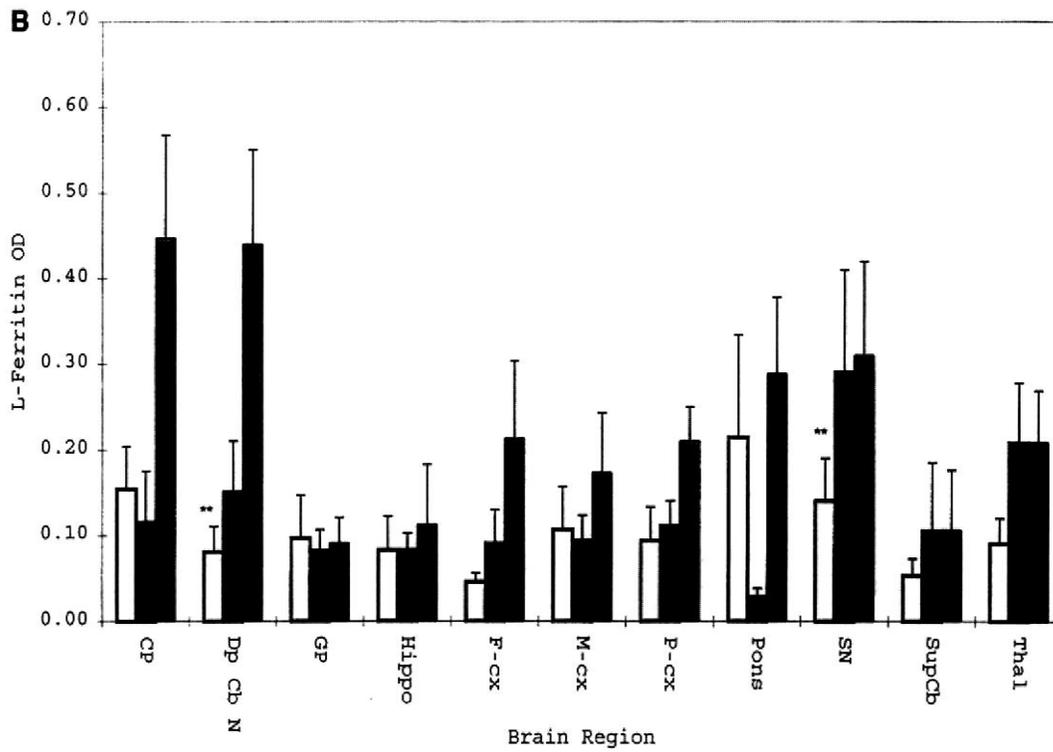
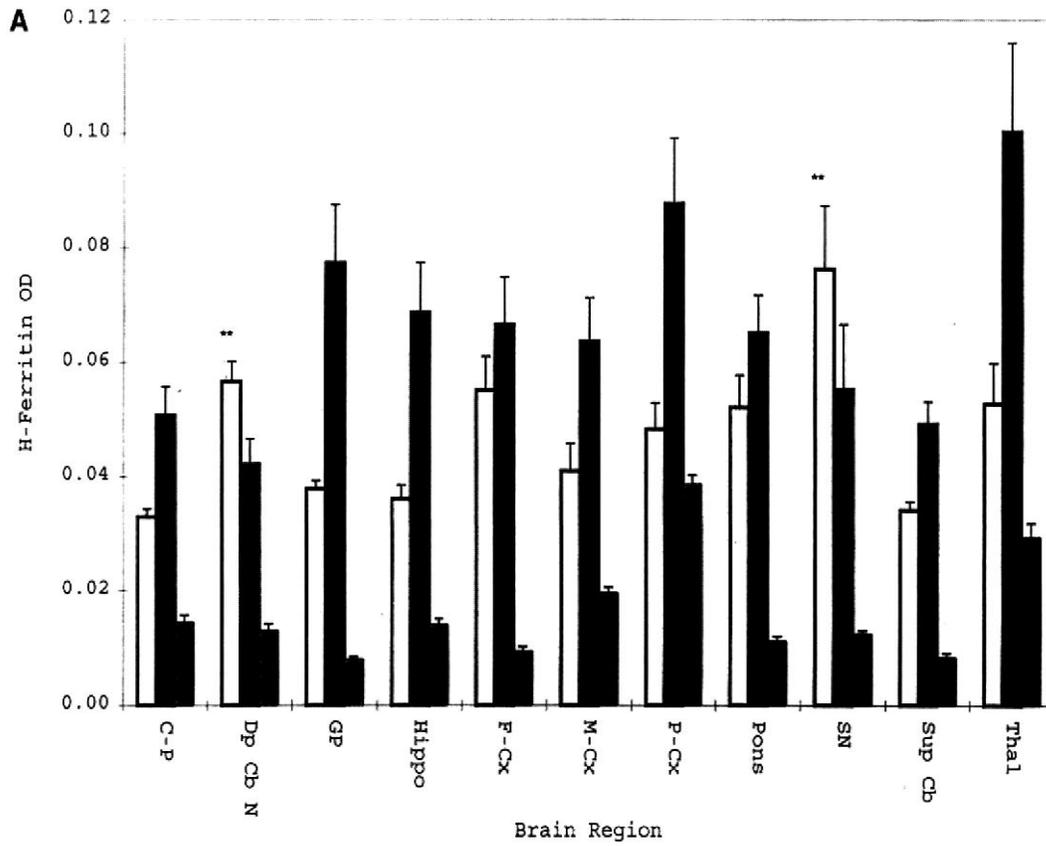
Results

Hematologic and liver non-heme Fe

Hb, Hct, and liver non-heme iron were significantly lower in ID pigs compared with CN animals ($P < 0.01$) (Table 1). Serum iron and Tf saturation were also significantly lower in the ID group compared with CN ($P = 0.03$ and $P = 0.02$, respectively) (Table 1). As would be expected, total iron binding capacity (TIBC) was significantly higher in the ID group than in the CN group ($P < 0.01$) (Table 1). Although the hematologic parameters of the CN piglets are much lower than usual adult levels, they are within normal limits for supplemented 28-day-old piglets.⁵

Cytosolic and microsomal brain Fe

Cytosolic and microsomal brain iron concentrations were similar in all brain regions within a treatment group, except deep cerebellar nuclei and superficial cerebellum in CN pig brains and caudate putamen in ID pig brains. Regional brain iron concentrations in microsomal and cytosolic fractions were significantly lower in ID piglets than in CN piglets ($P < 0.05$) (Figure 1A and Figure 1B, respectively). The mean iron concentration in ID microsomal regions averaged $25 \pm 4\%$ of CN brain regions except for superficial cerebellum and posterior cortex (66% and 54% of control, respectively). In contrast, cytosolic iron concentrations in ID piglet brain regions was much more variable, with an average of $44 \pm 38\%$ of CN iron concentrations. It is important to note that iron was distributed in a heterogeneous fashion (i.e., iron deficiency did not affect each region equally) ($P < 0.05$). Regional brain iron concentrations in both microsomal and cytosolic fractions of the adult brain tissue were higher than those of the neonatal pig ($P < 0.001$) (Figure 1A and Figure 1B, respectively) but distributed across the brain in a pattern similar to that of the younger animals.



(continued)

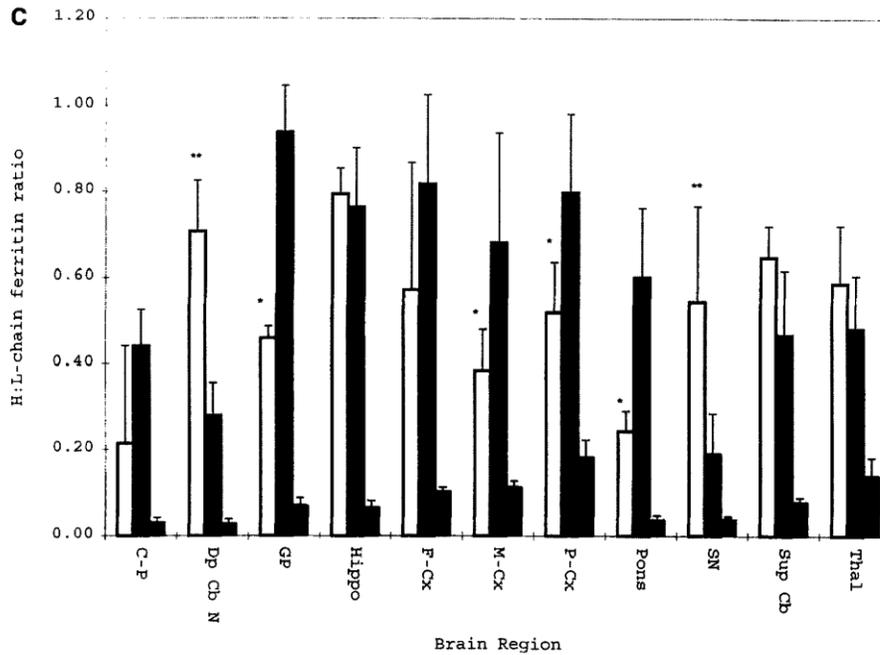


Figure 2 Regional brain H-chain (Figure 2A) and L-chain (Figure 2B) ferritin immunoreactivity and H-chain to L-chain ferritin ratios (Figure 2C) for iron deficient piglets (ID) (n = 5), control piglets (CN) (n = 5), and an adult pig (AD) (replicates = 6) determined in pooled microsomal and cytosolic fractions. Brain regions examined were caudate putamen (CP), deep cerebellar nuclei (Dp Cb N), globus pallidus (GP), hippocampus (Hippo), frontal cortex (F-Cx), medial cortex (M-Cx), posterior cortex (P-Cx), pons, substantia nigra (SN), superficial cerebellum (Sup Cb), and thalamus (Thal). Iron deficiency had an overall main effect of lowering brain regional H-chain immunoreactivity ($P < 0.05$), except in Dp Cb N and SN where ID levels were significantly higher than CN [$P < 0.05$; indicated by (**)]. Regional brain H-chain immunoreactivity of AD was significantly lower than CN ($P < 0.01$). Standard error bars represent replicate variation in AD and animal variation in ID and CN. (Figure 2B) Iron deficiency did not have an overall main effect on lowering brain regional L-chain ferritin, except in Dp Cb N and SN where ID levels were significantly lower than CN [$P < 0.05$; indicated by (**)]. Regional brain L-chain immunoreactivity of AD was significantly higher than CN ($P < 0.05$), except in GP, SN, Sup Cb, and Thal. Standard error bars represent replicate variation in AD and animal variation ID and CN. (Figure 2C) The ratio is H-chain to L-chain ferritin optical density (OD). Dunnett's procedure revealed a significantly lower ratio in GP, M-Cx, P-Cx, and Pons (*) and a higher ratio in Dp Cb N and SN (**) due to iron deficiency (ID) ($P < 0.05$). Regional brain H-chain to L-chain ferritin ratios of AD were significantly lower than CN ($P < 0.05$). Standard error bars represent replicate variation in AD and animal variation in ID and CN. (□ = ID; ■ = CN; ▒ = AD)

Brain ferritin

The immunoreactivity of brain regions to both H-chain and L-chain monoclonal antibodies is expressed as optical density (OD) (Figure 2A⁴ and Figure 2B, respectively). Ferritin was also examined as a ratio of H-chain to L-chain (H:L chain) ferritin (OD) (Figure 2C). Because cytosolic and microsomal ferritin immunoreactivity were not statistically different within brain regions ($P = 0.34$), subcellular fractions for samples were pooled from each brain region within a treatment group. H-ferritin immunoreactivity varied significantly from region to region ($P = 0.04$) with thalamus highest, followed by posterior cortex and globus pallidus in the CN piglets. Most regions had a lower immunoreaction to the H-chain ferritin antibody due to iron deficiency, except deep cerebellar nuclei and substantia nigra, which were significantly higher ($P < 0.05$) than control levels. All regions of the adult brain had a significantly lower immunoreaction to H-chain antibody ($P < 0.05$) (Figure 2A).

L-ferritin immunoreactivity varied significantly from region to region ($P < 0.05$) with substantia nigra having the highest, followed by thalamus and deep cerebellar nuclei in CN piglets. Interestingly, both substantia nigra and deep cerebellar nuclei of the ID piglets were 50% lower than CN levels and this difference was significant ($P < 0.05$). Adult L-ferritin immunoreactivity was significantly higher in caudate putamen, deep cerebellar nuclei, frontal, medial, and posterior cortical regions ($P < 0.05$) (Figure 2B).

The H:L chain ferritin ratio varied significantly from region to region ($P < 0.0001$) (Figure 2C). Dunnett's procedure revealed that iron deficiency caused a significant change in this ratio in six brain regions. In the ID piglets, globus pallidus, medial cortex, posterior cortex, and pons were significantly lower, whereas deep cerebellar nuclei and substantia nigra were significantly higher ($P < 0.05$). All brain regions of the adult pig had ratios significantly lower than CN ratios ($P < 0.05$) (Figure 2C). On average, approximately 70% to 80% of ferritin was H-chain in the piglets, whereas the adult ferritin was nearly 90% L-chain immunoreactive.

Brain transferrin

Because cytosolic and microsomal transferrin levels were not different within regions ($P = 0.65$), as with ferritin, subcellular fractions for samples were pooled from each brain region (*Figure 3*). There was a heterogeneous distribution of transferrin protein levels in the pig brain ($P = 0.014$). The response to iron deficiency was very small in most regions, although transferrin concentration in the caudate putamen and medial cortex was significantly higher ($P < 0.05$) in the ID pigs. In comparison to the levels in the neonatal pig brains, adult brain transferrin levels were significantly lower in most brain regions, except the posterior cortex ($P < 0.001$) (*Figure 3*).

Correlational analysis of liver iron and select brain regions

The relationship of liver non-heme iron, an index of systemic iron status, to regional brain non-heme iron concentration was investigated. Substantia nigra was the only brain region that showed a correlational relationship with liver microsomal iron ($r = 0.848$, $P < 0.05$) (*Figure 4A*). The caudate putamen is representative of the insignificant correlational relationship with most brain regions and liver [CP microsomal iron and liver microsomal iron ($r = 0.268$)] (*Figure 4B*).

Discussion

It is apparent from the hematologic data in the 1-month-old piglet that iron is rapidly utilized. Iron 100 mg on PND 2 was sufficient to keep Hct and Hb within normal range for young pigs⁵ and to have liver non-heme iron higher than in animals not injected with iron. However, TIBC in these pigs is very unsaturated and may be indicative of a rapidly growing animal in which iron supply is barely adequate.

It is apparent from the examination of the regional brain iron data that significant regional variations in iron exist in the pig as they do in other species.^{16,25} In contrast to the neonatal pig brain, regional brain iron distribution in the postweanling rat had much greater disparity.¹⁵ In other words, in the rat there was as much as a fourfold difference in iron concentration between regions (cortex versus hippocampus), whereas in the piglet there was a twofold difference between the regions with the lowest and highest iron concentrations (frontal cortex and deep cerebellar nuclei). Iron deficiency affected the rat brain differently than it affected the pig brain. For example, in the rat, the hippocampus experienced the most dramatic loss of iron due to iron deficiency with an up to 60% decrease; however, other regions were either unaffected by iron deficiency (substantia nigra) or diminished by only 10% to 20%. In the ID piglet, however, most of the regions lost approximately 50% of their cytosolic iron, but a few lost much less. The fact that frontal cortex and pons, and the microsomal fraction of the superficial cerebellum, were not as affected by iron deficiency as the other regions suggests a specific mechanism for iron retention in certain regions. This mechanism may involve regulatory control of ferritin, the trans-fen-in receptor, or perhaps other routes for the acquisition and control of brain iron. White matter is known to be higher in iron concentration than gray matter and special characteristics of these tissues may confer insensitivity to iron depletion.²⁶

There is either increased regional uptake of transferrin, increased regional production of transferrin, or some redistribution of transferrin in response to iron deficiency. This study did not examine regional transferrin receptor population, transferrin mRNA expression, or movement of transferrin. It can be assumed that all could be happening. In situ hybridization of transferrin mRNA in rats has indicated that the oligodendrocytes are primarily the cell in which transferrin mRNA is expressed.²⁷ It is thought that this expression is upregulated during development because myelination is being controlled by the oligodendrocytes. Because transferrin receptor expression and/or concentration was not measured, it must be assumed that increased regional transferrin receptor expression in combination with increased transferrin mRNA expression leads to increased amounts of transferrin in brain regions of ID piglets compared with CN piglets.

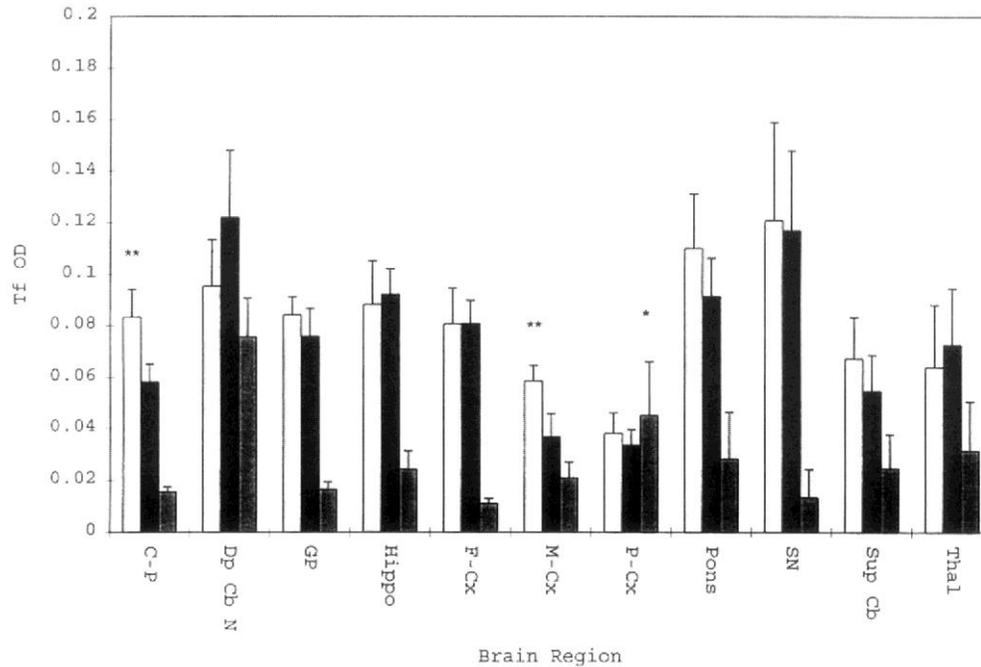


Figure 3 Regional brain transferrin (Tf) levels expressed as optical density (OD) for iron deficient piglets (ID) (n = 5), control piglets (CN) (n = 5), and adult pig (AD) (n = 1, replicates = 6). Transferrin was determined in pooled microsomal and cytosolic fractions. Brain regions examined were caudate putamen (CP), deep cerebellar nuclei (Dp Cb N), globus pallidus (GP), hippocampus (Hippo), frontal cortex (F-Cx), medial cortex (M-Cx), posterior cortex (P-Cx), pons, substantia nigra (SN), superficial cerebellum (Sup Cb), and thalamus (Thal). Regional differences in Tf between ID and CN were not statistically significant, except in CP and M-Cx [$P < 0.05$; indicated by (***)]. Regional Tf in AD compared with both ID and CN was statistically significant ($P < 0.05$), except in P-Cx, indicated by (*). Standard error bars represent replicate variation in AD and animal variation in ID and CN. (□ = ID; ■ = CN; ▨ = AD)

The "adaptation" to iron deficiency was not uniform throughout the brain. The caudate putamen increased transferrin levels in response to iron deficiency more than any other region, but still had absolute concentrations of transferrin similar to other regions. A major increase in transferrin concentration and a relatively smaller decline in iron content may be indicative of iron's important role for the developing caudate putamen. In the rat, the region that maintained the highest concentration of iron (hippocampus) during dietary iron deficiency, also had the highest transferrin concentration.¹⁵ Although the hippocampus did not show much particular sensitivity in the piglet model, the differences between species and the timing of iron deficiency may be very important sources of variation. Although most brain regions in the ID piglets had more transferrin than those in the CN piglets, some regions such as deep cerebellar nuclei, globus pallidus, hippocampus, and thalamus are more similar to CN levels.

Ferritin is an iron storage protein consisting of a combination of two subunits — H and L — whose synthesis is regulated by the intracellular content of iron through a well described iron-response-element-iron-response-protein regulatory sequence.^{13,27,28} H-chain predominant ferritin is generally associated with organs that are rapidly utilizing iron, whereas L-chain dominated ferritin is generally associated with organs that are storing iron.²⁸ Recently, it has been found that oligodendrocytes in 1-month-old piglets express H-chain ferritin but not L-chain ferritin and other cells in these brains contained little ferritin.²⁶ Because the present study used monoclonal antibodies against both H-chain and L-chain ferritin, a comparison between the adult brain and neonatal brain regional responses can be made. The immunoreactivity of both antibodies are expressed as a H:L chain ferritin ratio (*Figure 2C*). Because the neonatal pig brain regions had significantly more H-chain ferritin immunoreactivity than L-chain immunoreactivity, the H:L ratio was significantly higher than in adult brain regions. This difference may be indicative of a developmental process in which growing brain tissue expresses ferritin consisting of primarily the H-chain isoform.²⁶ The concept that different ferritin isoforms are developmentally regulated is not new.²⁹ The higher levels of L-chain ferritin in the adult pig brain regions are likely storage sites for the higher iron concentrations, although ferritin iron levels were not measured in any of these studies.

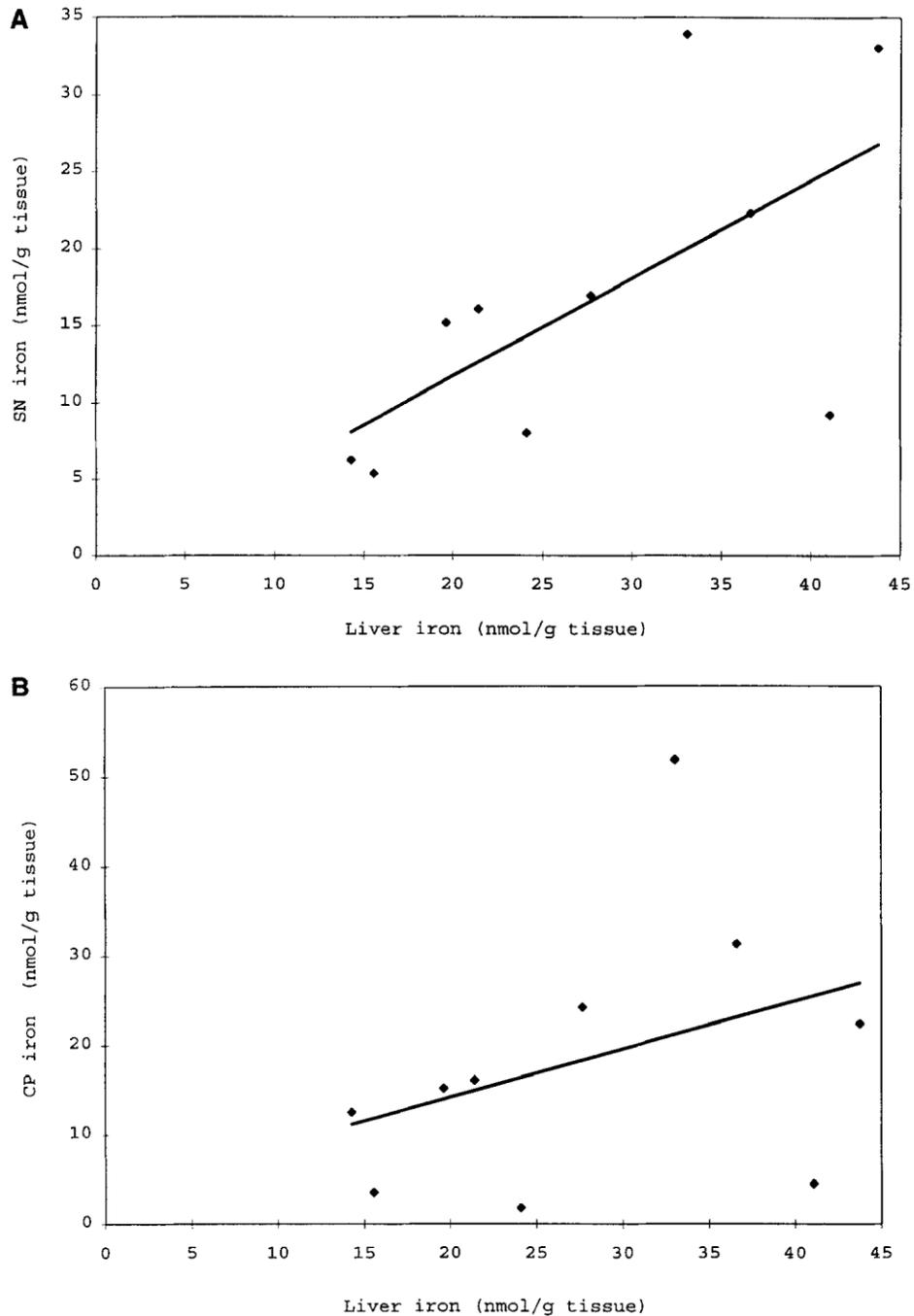


Figure 4 (Figure 4A) Scatterplot of liver microsomal iron versus substantia nigra (SN) microsomal iron in iron deficient (ID) (n = 5) and control (CN) (n = 5) piglets. The correlation was highly significant ($r = 0.848$, $P < 0.01$). (Figure 4B) Scatterplot of liver microsomal iron versus caudate putamen (CP) microsomal iron in iron deficient (ID) (n = 5) and control (CN) (n = 4) piglets. The correlation was not significant ($r = 0.268$, $P > 0.05$).

Iron deficiency altered the H:L chain ferritin immunoreactivity ratio in six brain regions. Deep cerebellar nuclei and substantia nigra both had significantly higher ratios, whereas globus pallidus, pons, medial, and posterior cortices had significantly lower ratios. These altered ratios may be characteristic of an alteration in a developmental process that has been hindered by iron deficiency, but more likely they are indicative of regional responses to iron deficiency. For instance, deep cerebellar nuclei is the brain region that has the highest iron content in both the CN and adult brains; therefore, it appears that iron may be important for this region. It is possible that H-chain ferritin may be expressed at a higher rate in this region during iron deficiency to retain the iron that is already present. However, because H-chain ferritin mRNA was not measured this is purely speculative.

In conclusion, the young growing pig and the young growing rat have a heterogeneous distribution of brain iron, transferrin, and ferritin.¹⁵ Although not identical between species, a consistent theme of regional control of rates of loss of iron is apparent. Similar data on developing humans are lacking; therefore, it is premature to assume these exact same events are happening to human infants during development. Nonetheless, the young piglet offers an clear opportunity to examine the relationship of rapid brain growth and development to systemic iron status.

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