Mild Maternal Iron Deficiency Anemia during Pregnancy and Lactation in Guinea Pigs Causes Abnormal Auditory Function in the Offspring1–3

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Abstract

Iron deficiency (ID) anemia (IDA) adversely affects different aspects of the nervous system such as myelogenesis, neurotransmitters synthesis, brain myelin composition, and brain fatty acid and eicosanoid metabolism. Infant neurophysiological outcome in response to maternal IDA is underexplored, especially mild to moderate maternal IDA. Furthermore, most human research has focused on childhood ID rather than prenatal or neonatal ID. Thus, our study evaluated the consequences of mild maternal IDA during pregnancy and lactation on the offspring’s auditory function using the auditory brainstem response (ABR). This technique provides objective measures of auditory acuity, neural transmission times along the peripheral and brainstem portions of the auditory pathway, and postnatal brain maturation. Female guinea pigs (n = 10/group) were fed an iron sufficient diet (ISD) or an iron deficient diet (IDD) (144 and 11.7 mg iron/kg) during their acclimation, gestation, and lactation periods. From postnatal d (PNd) 9 onward, the ISD was given to all weaned offspring. ABR were collected from the offspring on PNd24 using a broad range of stimulus intensities in response to 2, 4, 8, 16, and 32 kHz tone pips. IDA siblings (n = 3), compared with the IS siblings (n = 5), had significantly elevated ABR thresholds (hearing loss) in response to all tone pips. These physiological disturbances were primarily due to a sensorineural hearing loss, as revealed by the ABR’s latency-intensity curves. These results indicate that mild maternal IDA during gestation and lactation altered the hearing and nervous system development of the young offspring. J. Nutr. 141: 1390–1395, 2011.

Introduction

Iron deficiency (ID)9 anemia (IDA) is the most common single nutrient disorder in the world (1) with 1–2 billion people affected (2). IDA is a particularly important and prevalent health problem for pregnant women, because their nutritional demand increases in response to fetal development. Unfortunately, the diets of many pregnant women do not meet this increased iron requirement. Consequently, pregnant women are at high risk for developing IDA. It is thought that 50% of all pregnancy-related anemia cases are the result of ID (3). It is not just a poor country problem, since the prevalences of pregnant women experiencing anemia in developing and industrialized countries are over 50% (4) and 18% (5), respectively.

Both maternal (fetal) and childhood ID are major problems, because iron plays an important role in brain development. For example, childhood ID is associated with long-lasting behavioral (6), cognitive, motor, and language deficits despite subsequent iron therapy (7,8). Behavioral regulation also seems affected in the primates (8). However, the impact of ID during the prenatal and early postnatal periods on the brain’s neurophysiological outcomes and the infant’s sensory and central nervous system development is not well understood and has not been extensively studied.

Some of the impact of maternal IDA on offspring brain and sensory maturation and function can be assessed using the auditory brainstem response (ABR). This noninvasive technique is based on a sensory-evoked potential extracted from the electroencephalogram. ABR provide objective measures of auditory thresholds (auditory acuity) and neural transmission times along the peripheral and brainstem portions of the auditory pathway (9). They are also used to assess postnatal brain maturation (10) and detect and differentiate between peripheral and central hearing disorders (9,11). As

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3 Supplemental Methods are available from the “Online Supporting Material” link in the online posting of this article and from the same link in the online table of contents at jn.nutrition.org.
4 Abbreviations used: ABR, auditory brainstem response; CHL, conductive hearing loss; Gd, gestational day; GdbD, gestational day before delivery; Hb, hemoglobin; Hct, hematocrit; HPd, habituation period day; ID, iron deficient; IDD, iron deficient diet; IDA, iron deficiency anemia; IS, iron sufficient; ISD, iron sufficient diet; L-I, latency-intensity; PNd, postnatal day; SNHL, sensorineural hearing loss.
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Materials and Methods

Animals and diet

Twenty-two female and 2 male Hartley guinea pigs (Cavia porcellus), aged 10 wk, were purchased from Charles River Laboratories and housed in a controlled environment of 22°C at Université de Moncton. Of the 22 initial females, 1 IDA female died from unknown reasons 12 d after the study’s start and 1 IS female did not get pregnant. A 12-h light exposure was established daily in the animal care facility with lights on at 0700 h. Females were placed into 1 of 2 large cages for 3 wk of acclimation and habituation where they were randomly assigned and distributed this important health issue by evaluating the impact of mild IDA, especially during the prenatal/early postnatal periods. Our study addressed this important health issue by evaluating the impact of mild maternal IDA during gestation and lactation on the offsprings’ brain and sensory functions by using the ABR to determine the presence and nature of the potential hearing loss. The guinea pig was used, because unlike smaller rodents, both guinea pigs and humans (26) have a critical period of cerebral development that occurs prenatally.

ABR procedure

Pups were tested on PNd24, a stage of development during which they present a well-developed ABR (27–31). Before data recording, each animal received an i.m. injection (100 mg/kg) of an anesthetic solution of ketamine/xylazine (2:1), allowing excellent ABR recording (32). When sedated, guinea pigs were placed on a circulating water heating pad (T/Pump500, Gaymar Industries) to maintain normothermia, because temperature can influence ABR (33). Rectal temperature was monitored (Data Therm, Geratherm Medical) every 2 min and corporeal thermic fluctuations were < ± 0.5°C. ABR were recorded as described in the Supplemental Methods.

FIGURE 1 ABR traces in response to 2 kHz tone pips of descending intensities from a representative IS pup (A) and an IDA guinea pig pup (B) with an elevated threshold. Latency includes a 1.0 ms acoustic transit time.
Blood and tissue samples
Considering the guinea pig’s 74-d gestational period, hematocrits (Hct) were measured on Gd27, Gd48, and Gd68 and at weaning (PNd9) to determine at which equivalent trimester the IDA manifested itself. Blood samplings were done by alternately clipping the toenails and capillaries from 1 of the posterior legs. Hct was assessed by blood centrifugation in micro-capillary tubes and read in a microhematocrit reader. Hemoglobin (Hb) was not evaluated prenatally due to the small blood volume collection to limit stress to gestating females. At PNd24, ABR tests were performed and pups were killed thereafter by decapitation. Dams were killed on PNd9. A blood sample from pups (PNd24) and dams (PNd9) was sent to Dr. Georges L. Dumont Hospital in Moncton for Hct and Hb measurements.

Statistical analyses
Results are means ± SEM. Data were analyzed using SPSS version 17.0 statistical software. ABR data were scored by 2 independent judges who did not know the experimental treatments. The initial inter-judge agreement was >99% and the judges solved disagreements by discussion. If they could not agree (one case), the 2 scores were averaged. Independent t tests assessed potential differences in maternal and birth outcomes between IS and IDA groups. Mortality and gender distributions were analyzed by 2 × 2 chi-square tests. A repeated-measures ANOVA (group × time) was performed on the dams’ Hct. Because offspring of a same litter share genetic material with their mother, data (weight, Hct, Hb, ABR, and temperature) were transformed by computing a single composite variable described as sibling. In all analyses, sex was assessed as a within-sibling variable. Because not all dams gave birth to a male and a female, analyses were based on 9 dams (4 IDA and 5 IS) and 25 pups (9 IDA and 16 IS, i.e. 4 and 5 siblings, respectively).

The effect of paternity was tested and found to be not significant and so was not further analyzed. Pearson correlation coefficients were calculated for offsprings’ Hct and Hb data on PNd24 for each gender. These results confirm that mild IDA was induced.

Hematological data
Hct values were available for analysis from 6 IDA and 9 IS dams. The IS group maintained normal Hct (0.37–0.48) for adult guinea pigs (38) throughout gestation, whereas in the IDA group, the Hct decreased to a level that was nearly suboptimal (38) to gestating females. At PNd9, dams’ Hct (Fig. 2) and Hb concentrations (IDA, 100 ± 4 g/L; IS, 135 ± 4 g/L) decreased as expected after delivery, with values in the IDA group being below normal (38), and below the IS group values (P < 0.001 for Hct and Hb). These results confirm that mild IDA was induced in the IDA group. Hct and Hb siblings as a function of sex were strongly correlated (r = 0.88 and 0.95 for males and females, respectively; P < 0.001 for pooled groups) and the measures were combined into the doubly MANOVA. IDA and IS siblings had similar Hb [120 ± 3 and 120 ± 3 g/L, respectively; normal: 110–152 g/L (38)] and Hct values (0.36 ± 0.01 and 0.35 ± 0.01, respectively) on PNd24.

ABR
Thresholds (auditory acuity). IDA siblings had higher ABR thresholds (i.e. worse auditory acuity) than the IS siblings (P = 0.04) (Fig. 3) and extreme tone pip frequencies were associated with higher ABR thresholds (frequency, P < 0.001). The ABR tracings at 2 kHz exemplify the shift in thresholds (Fig. 1); the IS pup had an ABR present at 25 dB, whereas the IDA pup had an

Results
Maternal and offspring outcomes
At the beginning of the habituation period d (HPd) 1, females in both groups had similar body weights (Table 1). However, a delayed acceptance of the diet by the IDA females resulted in lower body weights for the latter at the end (HPd21) of the habituation period (day, P < 0.001; group × day, P = 0.05). IS females also had a greater body weight than IDA females (P = 0.005) during the gestational period (P < 0.001). Maternal weight gains and gestation length were similar in both groups between Gd1 and Gd before delivery (GdBd), indicating that the greater body weight of the IS females during the gestational period was the result of the delayed acceptance of the diet by the IDA females during the HP. Litter size, number of pups alive, pre-/postnatal survival, and gender distribution did not differ between the groups.

Sibling weights
As expected, both groups gained weight from PNd1 to PNd24 (P < 0.001). There was no difference between groups, not interaction. However, males weighed more than females (P = 0.04).

Hematological data
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Data are means ± SEM; n = 10 dams or 5 (IS) and 4 (IDA) siblings.

2 Refer to the subsection “Maternal and offspring outcomes” in “Results” for detailed ANOVA group × sex × day as repeated measure (HPd1-HPd21 and GdBd) analyses.

3 Refer to the subsection “Sibling weights” in “Results” for detailed ANOVA group × sex × day as repeated measure (PNd1-PNd24) analyses.
ABR at 40 dB, representative of the threshold elevation of ~15 dB in the deficient group.

**ABR L-I curves.** At the 32 kHz tone pip frequency (Fig. 4), 5 of 15 IDA (none were from the same sibling) offspring traces (33%) that were in the normal range in response to the highest stimulus intensities increasingly departed from normalcy as stimulus intensity progressively decreased. This proportion of abnormal L-I curves was different from all IS pups, which were in the normal range (*P*, 0.01). Only 1 of the 22 IS pups (4.5%) presented such an abnormal pattern. There were similar results in response to the 16 and 2 kHz tone pip condition where 20% (*P*, 0.05) and 13.3% (*P*, 0.08) of the IDA pups were affected. These L-I patterns suggest a mild recruitment-type SNHL. None of the IS pups presented abnormal curves at these 2 frequencies.

**Corporal temperature**

During the ABR procedure, body temperatures were normothermic and nearly identical for the IS (37.9 ± 0.2°C) and the IDA siblings (37.5 ± 0.2°C). Temperatures did not vary as a function of frequencies (i.e. time). Therefore, we did not control for this potential confounding effect in the analyses.

**Discussion**

The objective of this study was to investigate the effects of mild IDA during pregnancy and lactation on the offsprings’ brain and sensory function. Maternal IDA was induced by feeding guinea pig dams an IDD during the entire gestation until weaning. Our main finding was that this mild dietary condition had adverse effects on ABR outcome variables, consistent with a mild SNHL in the IDA offspring.

We reached our objective to induce maternal IDA and in fine to mimic mild conditions that frequently appears in pregnant women from industrialized countries. A low acceptance of the IDD during the acclimation period postponed the weight gain of IDA dams. However, pregnancy weight gain was not significantly different between groups. Birth weights were significantly lower for IDA siblings, but this was not a confounding variable in the ABR. Although Hct values were slightly below normal and given that the pups were in an anabolic period of life, the lower Hct values were not unexpected for young guinea pigs [as it is for children (39–41)] and Hb concentrations were within normal limits. Therefore, at the time of ABR testing, our IDA siblings were IS with normal hematological outcomes.

The present study demonstrated that moderate maternal IDA during pregnancy and lactation had a negative impact on the auditory acuity of the progeny via an elevation of their ABR thresholds (hearing loss). This deleterious effect was observed in response to all tone pip frequencies, suggesting that IDA may affect parts of the cochlea indifferently. Thresholds became better (lower) as frequencies gradually descended from 32 to 2 kHz, confirming that guinea pigs have a maximum auditory sensitivity in the lower frequencies (38).

A few studies have explored the effect of adult IDA on human auditory acuity with mixed results. For instance, IDA among 42 young adults (27.5 ± 9.7 y) did not support any relation with cochlear dysfunction using pure-tone audiometry and distortion product otoacoustic emission that evaluates outer hair cell function (42). In contrast, lower concentrations of serum ferritin, Hb, and iron were found in the RBC of 224 adults with hearing loss (43), suggesting a relation with ID. In adult IDA rodents, findings were also mixed, with absence of auditory...
sensitivity reduction (44), whereas cochlear lesions mainly involving outer hair cells (45), elevations of auditory thresholds (46), and reductions of spiral ganglion cells and stereocilia reflecting damage to the organs of Corti and the induction of a SNHL (46) were observed. Our results are consistent with the main finding of the latter study suggesting that ID can cause SNHL. However, unlike the above studies where IDA was examined during adulthood, our study was novel in that our dams were exposed to IDA during pregnancy and lactation and their progeny were studied.

To differentially diagnose either a CHL (middle ear impairment) or a SNHL (cochlea and/or auditory nerve), L-I curves of IDA pups were examined and were consistent with SNHL. We do not know if the SNHL is permanent or temporary (a developmental delay). A long-term, follow-up study is needed to answer this question. Even if the SNHL were to disappear in young adulthood, such animals could have increased risk for presbycusis (age-related hearing loss) and other morbidities in old age (11).

Several potential mechanisms may underlie the observed auditory abnormalities. Cellular impairment can be caused by an increase in reactive oxygen species in the inner ear (44), resulting in cochlear tissues damage (45). If the cochlea per se is physiologically affected, despite an unaltered auditory nerve pathway, this will convey erroneous information with elevated thresholds as a consequence. A decrease in neuronal surface proteins related to ID may also influence proliferative capacity and neuritic outgrowth, potentially leading to abnormal neuromediator release (47), thereby impairing auditory function. Pre- and postnatal ID could also induce phenotypic modifications of dendrite structures in rat hippocampus (48) or of animal behaviors (49). Severe postnatal ID in rodents affects neuromediator transporter ligand binding at multiple cerebral sites. Surprisingly, prepulse inhibitory mechanisms were not altered, whereas the acoustic startle response exhibited significant reductions and increased latencies to response were measured in ID females (50). Finally, ID might be implicated in epigenetic modifications and subsequently neurodevelopmental disorders in offspring (51,52).

In young mice, gestational ID (with or without repletion) significantly modified the lipid biochemistry of myelin (19) and cerebral fatty acids (24,25) during development and, as we demonstrate here, neurophysiological functions. Whether these 2 outcomes are related remains to be determined. However, a deficiency or an excess of dietary (n-3) fatty acids during gestation and lactation adversely affected auditory acuity in pups (28) with a persistence into young adulthood (53) and old age (11). Thus, we conclude that the alterations of the ABR we observed are likely the result of a concomitance of several mechanisms and that future investigation will be required to determine which mechanisms are involved.

Our results add to previous work and are relevant in several aspects. 1) We selected an animal model that has a critical period of cerebral development that occurs prenatally, as in humans. 2) We exposed our dams to IDA during prenatal and lactation periods, rather than during childhood or adulthood, in fine mimicking conditions that commonly occur in humans. 3) We induced a mild, rather than severe, maternal IDA. 4) We included sex as a within-sibling variable in all our statistical models. 5) We assessed broad ranges of tone pip frequencies and stimulus intensities to extensively define the treatment effects and their location. 6) We developed L-I curves and found that SNHL occurred in many of the ID offspring. 7) Finally, we found that the elevated ABR threshold effects occurred even though blood Hct and Hb had normalized, eliminating these 2 confounding factors.

In conclusion, mild IDA is associated with harmful developmental effects and the present neurophysiological study extends this knowledge. The health-related implication of our findings is that merely mild levels of maternal IDA during pregnancy and lactation can cause sensory and neurological morbidities in the offspring. This is a health concern, because there is a high prevalence of mild maternal IDA in developed countries. Despite strategies to counter potential ID (via nutritional recommendations and iron supplementation), 18% of pregnant women in industrialized countries are still ID. The present study on sensory and brain functions coupled with our previous observations on activity levels of offspring (25) suggest that consequences of moderate and even mild maternal IDA may be more detrimental than previously thought. The potential long-lasting effects of maternal IDA on the offspring’s auditory functions warrant further investigation as does the elucidation of the mechanisms by which it is involved in nervous system development. Such observations, if confirmed in humans, should lead to a greater impetus for public health education programs.

Acknowledgments
J.-L.J., F.M.R., M.W.C., S.F., and M.E.S. were involved in the experimental design; J.-L.J. conducted research; J.-L.J. and S.F. performed statistical calculations; J.-L.J., M.W.C., and S.F. analyzed the data; J.-L.J. wrote the paper; and J.-L.J., F.M.R., M.W.C., S.F., and M.E.S. contributed to the review and editing of the manuscript. All authors read and approved the final manuscript.

Literature Cited
ERRATUM


All symbols for micrograms were inadvertently removed from the PDF of this article before online publication. The online PDF has been corrected by the publisher to reflect this change.

In addition, in the third paragraph on page 134, under the heading “Plasma Il-6 levels in mice with CIA,” the following change should be made:

In unimmunized mice, plasma IL-6 levels did not differ among the 3 dietary treatment groups (Fig. 4B). In CII-treated mice fed the WBM diet, the mean concentration of plasma IL-6 was 2.7 times that of mice fed the control diet (P < 0.05). After CII injection, mice fed the SM diet had a 1.3-fold increase in plasma IL-6 levels compared with those of immunized mice fed the same diet (P < 0.05). No such increase was observed in mice fed the control and WBM diets.

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Please note the following corrections:

An error occurred on page 1392, in the last sentence of the first paragraph of the statistical analyses. The sample value for IDA pups is incorrect and indicated that “analyses were based on 9 dams (4 IDA and 5 IS) and 25 pups (9 IDA and 16 IS, i.e., 4 and 5 siblings, respectively).” The corrected sentence should read: “analyses were based on 9 dams (4 IDA and 5 IS) and 27 pups (11 IDA and 16 IS, i.e., 5 and 5 siblings, respectively).”

In the sixth sentence of the second paragraph of the statistical analyses, “IDA and IS siblings were reduced to 3 and 5 …” should read “IDA and IS siblings were reduced to 4 and 5…”

Corrections should be also applied to the abstract on page 1390, next to the last sentence: “IDA siblings (n = 3), compared with the IS siblings (n = 5), had significantly elevated ABR thresholds” should read “IDA siblings (n = 4), compared with the IS siblings (n = 5), had significantly elevated ABR thresholds.”

In the Figure 3 legend on page 1393, the sample value for IDA pups should read as n = 4, instead of n = 3.

This error does not affect the outcomes and findings.

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