Maternal Iron Deficiency Alters Essential Fatty Acid and Eicosanoid Metabolism and Increases Locomotion in Adult Guinea Pig Offspring

Caroline P. LeBlanc, Sylvain Fiset, Marc E. Surette, Huguette Turgeon O’Brien, and France M. Rioux

Abstract

Iron deficiency (ID) is the most prevalent worldwide nutritional deficiency. Groups at risk of developing ID anemia are infants and pregnant women, even in industrialized countries. Our goal in this study was to evaluate the long-term consequences of maternal ID on the offspring’s fatty acid and eicosanoid metabolism, behavior, and spatial memory. Female guinea pigs consumed iron-sufficient (IS) and –deficient (ID) diets for 14 d before mating and throughout pregnancy and lactation. Dietary iron restriction resulted in ID in pregnant females. On postnatal d 9, all offspring (ID and IS) were weaned to the IS diet and at 42 d, all offspring were iron replete. Locomotion was tested in pups on postnatal d 24 and 40 and spatial memory from d 25 to 40. Pups from the ID group were significantly more active in the open field at both times of testing, whereas spatial memory, tested in a Morris water maze, was comparable in both groups. On postnatal d 42, liver, RBC, and brain fatty acid composition were measured. Dihomogammalinolenic [20:3(n-6)], docosapentaenoic [22:5(n-3)], and docosahexaenoic [22:6(n-3)] acid contents were significantly higher in brain phospholipids of offspring born to ID dams. Prostaglandin E2 and F2α concentrations were also significantly higher in brains of offspring born to ID dams. This demonstrates that moderate ID during gestation and lactation results in alterations of brain fatty acid and eicosanoid metabolism and perturbation in behavior in adult offspring.

Introduction

In early brain development, iron is involved in tissue oxygenation and energy metabolism (1). Iron is also essential to neurotransmitter synthesis and myelination of the central nervous system (CNS) (2) and as a cofactor to enzymes involved in brain development (3). Because iron deficiency (ID) during this critical period could cause permanent unfavorable effects on CNS development, it is crucial that the brain acquires an adequate iron supply during development (4). Unfortunately, many women do not maintain an adequate iron status during pregnancy because of increased requirements and intakes below recommendations (5). Infants and pregnant women are therefore at high risk of developing ID even in industrialized countries (6–8).

Although clinical studies have shown that maternal ID has a strong impact on infant iron status at birth (9,10) and during the first year of life (8,11,12), the long-term consequences of maternal ID on the progeny are not well known. Indirect evidence using IQ scores in human infants suggests that inadequate iron status during pregnancy can lead to delays in human infant cognitive and psychomotor development (13). Animal studies also support the potential negative effects of maternal ID on the infant’s CNS development. Severe ID during pregnancy in rats results in decreased activity in the young progeny (14) and sensorimotor deficits that are enduring (15). Similarly, marginal ID during pregnancy and lactation in mice is associated with altered functional motor development (16), which is not corrected by adequate iron intake after weaning (17).

Like iron, the long chain (n-6) and (n-3) PUFA and their metabolites, the prostaglandins (PG), are essential to brain...
development. Docosahexaenoic acid (DHA) and arachidonic acid (AA) are important structural fatty acids in the brain (18) that are found in high concentrations. DHA plays a functional role in neural and visual processes whereas AA is required to maintain normal growth and function of the vascular system (19). PG concentrations and the expression of their receptors are elevated in the newborn brain (20), where they regulate cerebral blood flow and nitric oxide synthase during the pre- and postnatal periods (21). PG receptors are expressed in astrocytes and oligodendroglial cells and PG are thought to be involved in the regulation of myelin production and oligodendroglial cell differentiation (22).

The biochemical mechanism by which maternal ID affects the CNS development in the progeny is unknown. However, ID has been linked to altered tissue PUFA profiles (23–27). Iron is a cofactor for the desaturases that are required for the synthesis of long-chain PUFA from their 18-carbon precursors. Reduced amounts of AA were reported in the plasma (25) and in liver phosphatidylcholine (PC) (27) of adult rats fed ID diets. Similarly, liver and serum AA were lower in rat pups born to dams fed an ID diet throughout pregnancy and lactation (28,29), whereas reduced AA and DHA levels were measured in brain myelin of mice pups born to dams fed a diet containing marginal amounts of iron (29). Whether these effects are reversible by replenishing iron stores in the progeny is uncertain. Additionally, the effects of ID on brain PG biosynthesis are unknown even though the cyclooxygenases (COX) I and II that are responsible for their biosynthesis are iron-containing enzymes (30).

In this study, we examined the consequences of moderate ID (31) during gestation and lactation on behavior and spatial memory in adult offspring of guinea pigs. The level of ID induced in pregnant females is considered to be moderate in guinea pigs and in human pregnant women. Tissue fatty acid composition and PG levels were also measured. The majority of previous studies investigating the effects of ID on brain development have used rats as the animal model. However, CNS development in rats occurs mainly during the postnatal period (32). We therefore selected guinea pigs, because this animal model more closely approximates the timing of prenatal brain development of humans (33) and is thus susceptible to altered development as a result of maternal dietary deficiencies.

**Materials and Methods**

**Guinea pigs and diets.** Nineteen female and 2 male Hartley guinea pigs (75 d old) were purchased from Charles River and were housed in the animal care facility at Université de Moncton, Edmundston campus, in a temperature-controlled environment (22°C) on a 12-h-light/-dark cycle with lights on at 0700. The total number of successful pregnancies in each group was 7 for the iron-sufficient (IS) group and 9 for the ID group. The other females refused to eat the diet, did not become pregnant, had a miscarriage, or had stillbirths. The total number of offspring was 15 for the IS group and 18 for the ID group.

Diets were purchased from Harlan Teklad. The iron contents of the IS and ID diets were 145 and 8.44 mg iron/kg feed, respectively (Table 1). Both IS and ID diets had the same fatty acid profile. The main fatty acids in the diet were linoleic acid [18:2(n-6)], oleic acid [18:1(n-9)], and palmitic acid 16:0, accounting for 58.9 ± 0.6%, 26.4 ± 0.3%, and 10.7 ± 0.1% of total fatty acids, respectively. Small amounts of stearic acid [18:0] and α-linolenic acid [18:3(n-3)] were also present at levels of 1.8 ± 0.03% and 1.0 ± 0.01% of total fatty acids, respectively. Diets were consumed ad libitum along with fresh water. Food intake was recorded daily and body weight every second day. The research protocol was approved by the Université de Moncton animal care committee.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of IS and ID guinea pig diets1</th>
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<tbody>
<tr>
<td></td>
<td>IS</td>
</tr>
<tr>
<td>Casein</td>
<td>300</td>
</tr>
<tr>
<td>l-Arginine HCl</td>
<td>4</td>
</tr>
<tr>
<td>Sucrose</td>
<td>366.3</td>
</tr>
<tr>
<td>Cellulose</td>
<td>150</td>
</tr>
<tr>
<td>Corn oil</td>
<td>75</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin mix (4000D)</td>
<td>10</td>
</tr>
<tr>
<td>Minerals1</td>
<td>59.9</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>0.735</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3</td>
</tr>
<tr>
<td>Choline dihydrogen citrate</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.014</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Protein macronutrient contribution of IS and ID diets (%): protein, 33.7; carbohydrate, 45.7; lipid, 20.6.

1 Containing the following (g/kg vitamin mix): p-aminobenzoic acid, 11.01; ascorbic acid, 101.66; biotin, 0.044; vitamin B12, 2.97; calcium pantothenate, 6.61; choline dihydrogen citrate 349.69; folate, 0.198; myoinositol, 11.01; menadione, 4.96; niacin, 9.91; pyridoxine HCl, 2.20; riboflavin, 2.20; thiamin HCl, 2.20; vitamin A palmitate, 3.96; cholecalciferol, 0.441; vitamin E acetate, 24.22; corn starch 466.69.

**Experimental design.** Females were randomly distributed into 2 groups and consumed their respective diets (ID or IS) for 14 d before mating and continued throughout gestation and lactation. For each dietary group, 1 male was placed with 5 females in a large mating cage for 23 d. A comparable number of females per dietary group were mated with the same male. After mating, females were then separated and placed individually in cages. On postnatal d 9, pups were weaned and all pups were fed the IS diet.

**Exploratory behavior in the open field.** At 24 and 40 d of age, pups were tested in an open field to assess their spontaneous locomotor activity, as previously reported in mice (34). The apparatus consisted of a 20-cm-high, 1-m × 1-m enclosure made of white Plexiglas. The bottom of the enclosure was covered with 3 cm of pine wood chips. On the days of testing, each pup was placed in the open field and activity was recorded via an overhead video camera for 15 min. The behavioral measures recorded were the total number of peripheral and central square crossings, the total number of movements (i.e. starts and stops), and the mean duration of each movement.

**Morris Water Maze.** The Morris Water Maze (MWM) consisted of a circular pool (165 cm diameter, 75 cm deep) filled with water (47 cm deep; temperature 23–25°C) made opaque with the addition of non toxic white paint. A circular escape platform (25 cm diameter, 45 cm high) made of transparent Plexiglas was submerged 2 cm below the water surface and located in the center of 1 of the 4 pool quadrants. This spatial memory task was largely adapted from Dringenberg et al. (35), who first validated the MWM in guinea pigs. Starting on postnatal d 25, offspring were tested in the MWM. During the acquisition phase, guinea pigs were tested for 10 consecutive days with the submerged platform remaining in the same quadrant. The position of the platform among the 4 quadrants was randomly distributed across animals. On each day, guinea pigs received 1 block of 4 training trials. For each block, 4 randomized start positions from the 4 cardinal compass points were used. The guinea pig started a trial facing the wall of the pool and was allowed to swim freely for 45 s. If the guinea pig found the platform, it was left there for 15 s and then removed and dried to wait for next the trial. If the guinea pig did not find the platform after 45 s, it was manually placed on it for 15 s and then removed and dried to wait for the next trial. Time required to reach the
platform was recorded via a stopwatch and a camera mounted on the ceiling over the pool.

Five days after the end of the acquisition phase (postnatal d 39), the platform was removed and each guinea pig was allowed to swim freely in the pool for 60 s. Twenty minutes after this retention trial, the same guinea pig was placed in the pool again, but this time the platform was relocated to the opposite quadrant of the maze. For this reverse quadrant platform test, the guinea pigs received 1 block consisting of 4 trials on postnatal d 39 and 1 block of 4 trials on postnatal d 40. This test was performed to determine whether pups transferred learning from one position to another.

**Tissue and blood collections.** At the age of 42 d, the pups were anesthetized with a solution of 10:1 ketamine/xylazine (1 mL/kg body weight) and killed by decapitation. Two 1-mL blood samples were then collected in EDTA-anticoagulated tubes. One tube was sent to the Edmundston Regional Hospital in Edmundston, New Brunswick, for complete blood count [hemoglobin (Hb), hematocrit (Hct), and mean cell volume (MCV)] using a Beckman-Coulter Ac-T diff2 Hematology Analyzer. Spectrophotometry was used for Hb and aperture impedance was used for MCV and RBC. Hct was calculated by multiplying MCV by RBC and dividing by 1000. The other blood sample was immediately put on ice for preparation of RBC. The plasma was separated by centrifugation at 10,000 \( \times \) g; 15 min at 4°C and RBC were washed twice with 3 mMol/L EDTA 0.9% saline solution. Aliquots were stored at −70°C until further analysis. The brain and left lobe of the liver from each pup were immediately dissected, weighed, and then frozen in liquid nitrogen and stored at −70°C until further analyses.

**Homogenization and tissue preparation.** Brain and liver tissues were homogenized in a Potter apparatus. One gram of tissue was homogenized in 9 mL of ice-cold homogenization buffer containing 50 mMol/L potassium phosphate, pH 7.4, and 0.25 mol/L sucrose. For brain tissues, the homogenization buffer also contained indomethacin (10 mg/L) (Alfa Aesar). The supernatant was obtained from the homogenate following centrifugation at 12,000 \( \times \) g; 10 min at 4°C. Protein content was determined using a modification of the Lowry method (36).

**Fatty acid quantification.** Total lipids of brain and liver homogenates and of RBC were extracted with chloroform (37). For brain lipids, phospholipid (PL) classes [PC, phosphatidylinositol (PI)/phosphatidylserine, and phosphatidylethanolamine (PE)] were separated by HPLC as previously described (38).

FAME were prepared from brain PL classes (PE, PI/ phosphatidylserine, and PC) and from total lipids extracted from liver and RBC following the addition of 1,2-dihexadecanoyl sn-glycero-3-phosphorylcholine (Biolynx) as an internal standard and were analyzed by GC with flame ionization detection as previously described (39).

**Brain COX II western blotting.** Brain homogenate supernatants were heated at 100°C for 5 min in Laemmli sample buffer (40) and separated by SDS-PAGE on a 10% gel. The proteins were then electrophoretically transferred to a polyvinylidene difluoride membrane (Millipore) and incubated for 30 min in Tris-buffered saline (TBS)-Tween [0.15% (v/v) Tween-20 in TBS, pH 7.6] containing 5% (w/v) nonfat dry milk. After washing, the membrane was incubated with an anti-COX II (Cayman Chemical) diluted 1:200 in TBS-Tween containing 0.02% sodium azide (v/v) for 60 min. Membranes were then washed and incubated for 45 min in TBS-Tween containing horseradish peroxidase-conjugated goat anti-mouse (1:45,000 dilution, Jackson ImmunoResearch Laboratories). Membranes were then washed and developed using Super-signal West Femto Substrate (Pierce) with detection using an Alpha Innotech Fluorchem imager. Optical densities of bands were measured using Alpha Ease Fluorchem Software 4.1.0.

**Measurement of PGE2 and PGF2a.** PGE2 and PGF2a concentrations were measured in brain supernatant using PGE2 and PGF2a Correlate-EIA kits (Assay Designs) according to the manufacturer’s protocol. All samples were analyzed in duplicate and plates were read at 405 nm using a Varioskan (Thermoelectron).

**Statistical analysis.** Data were analyzed using SPSS version 14.0, 2005 software. Two-way repeated-measures ANOVA were used to identify significant differences between groups and sessions in the open field and the MWM and to compare offspring’s body weight of both groups at birth and d 39. Significant main within-subject effects were followed by Bonferroni tests. t tests were used for all other comparisons. Values in the text are means ± SEM and the level of significance was set at \( P < 0.05 \).

**Results**

**Food intake, iron status, and weights.** Food intake during gestation did not differ among ID and IS groups; the IS group consumed 31.5 ± 1.5 g/d and the ID group consumed 31.0 ± 1.7 g/d. Dietary restriction of iron during pregnancy and lactation resulted in ID in guinea pig dams, because Hb, Hct, and MCV were significantly lower in the ID group (Table 2). The mean Hb for dams of the ID group was well below the normal range for Hb in guinea pigs, which usually varies between 110 and 140 g/L, with a mean of 125 g/L (31). Iron status did not differ between the 2 groups of pups at 42 d. Offspring’s body weight increased from birth to d 39, but there was no significant difference between groups or time x group interaction. On postnatal d 42, offsprings’ brain and liver weights did not differ (Table 2).

**Exploratory behavior in the open field.** Groups behaved differently in the open field. The ID group was significantly more active than the IS group at both 24 and 40 d postpartum. In each session, the ID group had significantly more central and peripheral square crossings and mean number of movements. There was no significant effect of sessions and no interaction. The duration of each movement significantly decreased from d 24 to 40, but there was no difference between groups and no interaction (Table 3).

**MWM.** During the acquisition phase of the MWM, escape latency gradually decreased from d 1 to d 10 of training (Fig. 1A), but there were no significant differences between offspring from the IS and ID groups nor was there a time x group interaction. In the retention trial (platform removed), guinea pigs spent more time in the target quadrant than expected by chance (15 s), but there were no diet group differences (data not shown), suggesting that both groups remembered the location of

**TABLE 2** Body and organ weights and iron status of guinea pig offspring and dams fed the IS and ID diets1

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>ID</th>
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<tbody>
<tr>
<td>Offspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>116.1 ± 4.7</td>
<td>100.1 ± 3.5</td>
</tr>
<tr>
<td>Postnatal d 39</td>
<td>358.1 ± 13.9</td>
<td>327.2 ± 10.9</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>4.7 ± 0.3</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>3.5 ± 0.0</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Hb (postnatal d 42), g/L</td>
<td>130.7 ± 1.9</td>
<td>127.7 ± 1.7</td>
</tr>
<tr>
<td>Hct (postnatal d 42)</td>
<td>0.39 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>82.3 ± 0.7</td>
<td>84.7 ± 0.6*</td>
</tr>
<tr>
<td>Dams (postnatal d 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>131.3 ± 2.0</td>
<td>88.4 ± 3.1*</td>
</tr>
<tr>
<td>Hct</td>
<td>0.40 ± 0.01</td>
<td>0.29 ± 0.01*</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>84.2 ± 1.1</td>
<td>62.3 ± 0.7*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7-9. (dams) or 15-18 (offsprings). *Different from IS, \( P < 0.05 \).
the platform 5 d after the end of training. Finally, offspring from both groups showed transferred learning when the platform was relocated in a new quadrant, as shown by a significant decrease in escape latency over the 2 test days (Fig. 1B). There were no diet group differences nor was there an interaction.

**Brain fatty acid composition.** ID induced during pregnancy and lactation had a lasting impact on the fatty acid composition of the pups’ brains (Supplemental Table 1). The concentration of total fatty acids did not differ between the IS and ID groups (data not shown). The content of total SFA in PI was lower in pups born to ID dams, mainly due to lower 18:0 content, whereas the 16:1(n-7) content was significantly lower in PE and PI of pups born to ID dams. Total proportions of PUFA in PE and PI were significantly higher in pups born to ID dams. Although no differences were measured in the percent of total (n-6) fatty acids, dihomogammalinolenic acid [20:3(n-6)] was significantly higher in all 3 PL classes, whereas 20:2(n-6) was significantly lower in PE and PI of pups born to ID dams. The most striking difference in fatty acid content between groups was in the (n-3) PUFA. Pups born to ID dams had significantly higher proportions of total (n-3) PUFA in PC, PE, and PI, with significantly higher levels of 22:5(n-3) and 22:6(n-3) in PE and PI (Table 4).

**Liver fatty acid composition.** The concentration of total fatty acids was higher in the IS group (67.4 ± 5.3 mg/g tissue) than in the ID group (53.4 ± 4.0 mg/g tissue) (P < 0.05). The 2 groups did not differ in total SFA, monounsaturated fatty acids, PUFA, or (n-3) or (n-6) fatty acids. However, the proportion of 16:0 was lower and those of 18:0, 20:1, and 20:2(n-6) were higher in pups in the ID group (Supplemental Table 2).

**RBC fatty acid composition.** The concentration of total fatty acids did not differ between the IS and ID groups (data not shown). The 2 groups did not differ in total SFA, monounsaturated fatty acids, PUFA, or (n-3) or (n-6) fatty acids. However, 18:1(n-7) and 20:3(n-6) were significantly lower in the ID group compared with the IS group (Supplemental Table 3).

**Brain COX II and PG levels.** Dietary restriction of iron during pregnancy and lactation resulted in significantly higher levels of PGE$_2$ and PGF$_{2\alpha}$ in brains of offspring born to the ID dams (Table 4). Consistent with the PG concentrations, COX-II protein levels tended to be higher in brains of pups born to ID dams compared with those born to IS dams (P = 0.10).

**Discussion**

In this study, the consequences of moderate maternal ID on behavior and spatial memory as well as on fatty acid and

**TABLE 3** Exploratory behavior in the open field of offspring of dams fed IS and ID diet during pregnancy and lactation$^1$

<table>
<thead>
<tr>
<th>Postnatal d 24</th>
<th>Postnatal d 40</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral square crossings, n</td>
<td>IS</td>
</tr>
<tr>
<td></td>
<td>IS</td>
</tr>
<tr>
<td></td>
<td>IS</td>
</tr>
<tr>
<td>Duration of each movement, s</td>
<td>IS</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, n = 15–18. *Different from IS pups, P < 0.05.

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**TABLE 4** Brain docosapentaenoic acid and DHA contents in PC, PE, and PI, brain PGE$_2$ and PGF$_{2\alpha}$ concentrations, and COX-II levels of offspring of dams fed IS and ID diet during pregnancy and lactation$^1$

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA, %</td>
<td>0.7 ± 0.05</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Docosapentaenoic acid, %</td>
<td>5.2 ± 0.03</td>
<td>6.3 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>4.1 ± 0.2</td>
<td>5.1 ± 0.3*</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>973 ± 50</td>
<td>1165 ± 70*</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>2080 ± 224</td>
<td>2375 ± 362*</td>
</tr>
<tr>
<td>COX-II, relative integrated density values</td>
<td>3.48 ± 0.31</td>
<td>4.88 ± 0.15</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, n = 15–18. *Different from IS pups, P < 0.05.
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to their role as precursors to the bioactive prostanoids. Indeed, PG concentrations and that of their receptors are elevated in the newborn brain compared with adults and PG are thought to be involved in regulating cerebral blood flow, myelin production, and oligodendrocyte differentiation in the perinatal period (21,22). Both COX-I and COX-II enzymes are expressed in the brain, although COX-II is primarily associated with changes in PG synthesis in the developing brain (3). In the present study, the concentrations of PGE₂ and PGF₂α were significantly higher in pups in the ID group. To our knowledge, no other studies have investigated the effects of maternal iron restriction on prostanoïd metabolism in the offspring, although we have shown that these differences are not apparent at weaning (58). With respect to arachidonate-metabolizing enzymes, others have measured a transient increase in the expression of the ALOX15 gene in the hippocampus of young pups born to dams fed an ID diet (64).

In conclusion, this study demonstrates that despite consuming an IS diet at weaning, moderate ID induced during gestation and lactation resulted in increased locomotor activity in an open field that is associated with altered PUFA and eicosanoid metabolism in the brain of offspring that persists into adulthood. Although it is difficult to make a causal association between these two outcomes, recent studies have linked dietary supplementation with PUFA to an amelioration in attention deficit hyperactive disorder-related symptoms in children (65,66).

In addition, these studies would elucidate whether the increased exploratory behavior observed in the offspring born to ID dams is associated with changes in PUFA and eicosanoid metabolism in specific brain structures. This study suggests that the guinea pig is likely to be an appropriate animal model for such investigations.

Acknowledgment
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Literature Cited