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Abnormal neurological responses in young adult offspring caused by excess omega-3 fatty acid (fish oil) consumption by the mother during pregnancy and lactation

M.W. Church [a,*], K.-L.C. Jen [b], D.A. Jackson [c], B.R. Adams [c], J.W. Hotra [a]

[a] Department of Obstetrics & Gynecology, Wayne State University, Detroit, MI 48201, USA
[b] Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48201, USA
[c] Department of Physiology, Wayne State University, Detroit, MI 48201, USA

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ABSTRACT

Consuming omega-3 fatty acids (ω-3 FA) during pregnancy and lactation benefits fetal and infant brain development and might reduce the severity of preterm births by prolonging pregnancy. However, diets that are relatively rich in ω-3 FA can adversely affect fetal and infant development and the auditory brainstem response (ABR), a measure of brain development and sensory function. We previously examined the offspring of female rats fed excessive, adequate or deficient amounts of ω-3 FA during pregnancy and lactation. The 24-day-old offspring in the Excess group, compared to the Control group, had postnatal growth retardation and poor hearing acuity and prolonged neural transmission times as evidenced by the ABR. The Deficient group was intermediate. The current study followed these offspring to see if these poor outcomes persisted into young adulthood. Based on prior findings, we hypothesized that the Excess and Deficient offspring would "catch-up" to the Control offspring by young adulthood. Female Wistar rats received one of the three diet conditions from day 1 of pregnancy through lactation. The three diets were the Control ω-3 FA condition (ω-3/ω-6 ratio ~ 0.14), the Excess ω-3 FA condition (ω-3/ω-6 ratio ~ 14.0) and Deficient ω-3 FA condition (ω-3/ω-6 ratio ~ 0% ratio). The Control diet contained 7% soybean oil; whereas the Deficient and Excess ω-3 FA diets contained 7% safflower oil and 7% fish oil, respectively. One male and female offspring per litter were ABR-tested as young adults using tone pip stimuli of 2, 4, 8 and 16 kHz. The postnatal growth retardation and prolonged neural transmission times in the Excess and Deficient pups had dissipated by young adulthood. In contrast, the Excess group had elevated ABR thresholds (hearing loss) at all tone pip frequencies in comparison to the Control and Deficient groups. The Deficient group had worse ABR thresholds than the Control group in response to the 8 kHz tone pips only. The Excess group also had ABR amplitude–intensity profiles suggestive of hyperacusis. These results are consistent with the Barker hypothesis concerning the fetal and neonatal origins of adult diseases. Thus, consuming diets that are excessively rich or deficient in ω-3 FA during pregnancy and lactation seems inadvisable because of risks for long-lasting adverse effects on brain development and sensory function.

1. Introduction

Omega-3 fatty acids (ω-3 FA) are "essential fatty acids" because it is necessary to acquire them through food consumption. Docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) are the chief ω-3 FA used as dietary supplements, usually in the form of fish oil. Maternal consumption of ω-3 FA during pregnancy and lactation can influence fetal and infant health and development. Regarding pregnancy, some studies reported that diets rich in ω-3 FA can increase birth weight and prolong pregnancy, thereby reducing the incidence and severity of preterm births and low birth weight infants [49,50,60,65]. Regarding lactation, the ω-3 FA in a mother's milk or in fortified infant formulas improve neurocognitive and visual development during the first year of life in comparison to infants who receive infant formula without ω-3 FA supplementation [2,9,33]. Thus, increasingly higher ω-3 FA doses are being recommended for pregnant women and nursing babies for advancing the health and development of preterm, low birth weight, and even normal infants [1,36,50].

Even though moderate amounts of ω-3 FAs are beneficial to the developing fetus and infant, there is new evidence that too much can be harmful. For example, several human studies found decreased gestational length and/or fetal growth retardation [25,26,48,51,58,68] and increased infant morbidity [68] from high fish oil consumption by the mother during pregnancy. Adverse effects from high ω-3 FA consumption by infants drinking formulas fortified with ω-3 FA include reduced body growth and head circumference [12,37,41], decreased blood arachidonic acid (AA) levels [8] and decreased verbal skills [40,62].

* Corresponding author. C.S. Mott Center for Human Growth & Development, 275 East Hancock, Detroit, MI 48201, USA. Tel.: +1 313 577 1184; fax: +1 313 577 1278.
E-mail address: mchurch@med.wayne.edu (M.W. Church).

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Animal studies report adverse effects as well. For example, prenatal and/or postnatal dietary supplementation with large amounts of ω-3 FA or a high ω-3/ω-6 FA ratio (due to low amounts of ω-6 FA) can result in reduced birth weight, postnatal growth impairment, increased pre- and postnatal mortality, decreased brain sizes, decreased AA levels, and/or abnormal neurobehavioral function [3,18,30,56,70] and abnormal retinal function and structure [38,74]. Studies using the auditory brainstem response (ABR), a measure of brain development and sensory function, found that high levels of dietary ω-3 FA supplementation in pregnant and lactating rats caused the offspring to have prolonged ABR wave latencies [19,30,59,67], delayed acoustic startle reflexes [30,59,67], reduced auditory acuity [18], and evidence suggesting impaired brain myelination [30] when tested as postweaning pups. Effects from pre- and postnatal diets that are rich in ω-3 FA are similar to those caused by ω-3 FA deficiency or a low ω-3/ω-6 ratio (due to ω-6 FA excess) which include impaired visual function [7,74], learning deficits, decreased brain weight and/or altered nerve FA composition [7,9,33,69,71] and ABRs indicating a faster aging brain in old age [6].

Unfortunately, the caveat that rich ω-3 FA diets can be harmful to the fetus and infant is being ignored [19]. Adverse fetal and neonatal environments can "program" the offspring for adult-onset health disorders [4,22]. Yet no one has investigated the possibility that nutritional toxicity from high levels of dietary ω-3 FA or a high ω-3/ω-6 ratio can cause fetal programming of adult-onset disorders, with the exception of a study which found altered body fat composition in adult rats born to dams receiving diets rich in ω-3 FA during pregnancy and lactation [35]. Thus, further research on the potentially harmful effects of prenatal and postnatal ω-3 FA excess and deficiency is rather important, particularly in terms of the long-term consequences.

With these issues in mind, our study's primary goal was to investigate the possibility that maternal consumption of diets that are excessively rich or deficient ω-3 FA during pregnancy and lactation cause long-term impairment of the offspring's nervous system as evidenced by the ABR. In prior studies, we found that our "ω-3 FA excess" condition caused postnatal growth retardation, hearing loss [18] and delayed neurotransmission times [19] in 24-day-old rat offspring. The current study followed these offspring longitudinally to see if these poor outcomes persisted into young adulthood. A recent study on ω-3 FA deficiency during pregnancy found that the rat offspring had abnormal ABRs as pups, which became normal in young adulthood, then became abnormal again in old adulthood [6]. Others have found this same age-dependent pattern in neurological outcomes following prenatal or neonatal exposure to various brain damaging substances [5,34,43,44,47,61,73]. Consequently, we hypothesized that our ω-3 FA excess and deficient offspring would show normalization of their ABRs during young adulthood.

2. Methods

2.1. Animals and diets

Wayne State University's animal investigation committee approved the procedures for this study. Institutional and NIH guidelines were followed.

Our procedures are detailed elsewhere [18,19]. Briefly, female Wistar rats, 10 weeks of age, were mated individually with male Wistar rats. The presence of a sperm plug was designated as gestational day one. The females were then placed in separate polycarbonate cages (25×45×20 cm) and randomly assigned to one of the three diet conditions starting from day 1 of pregnancy through the entire period of pregnancy and lactation. The three diets were the Control ω-3 FA condition (ω-3/ω-6 ratio ~ 0.14), the Deficient ω-3 FA condition (ω-3/ω-6 ratio ~ 0% ratio) and the Excess ω-3 FA condition (ω-3/ω-6 ratio ~ 14.0). The number of pregnant dams/litters in the Control, Deficient and Excess conditions were n=23, 31 and 22, respectively. The Deficient group had slightly more pregnant dams than the other two groups because of a better pregnancy success rate for unknown reasons. The Control diet contained 7% soybean oil. The Deficient ω-3 FA diet contained 7% safflower oil in place of soybean oil. The Excess ω-3 FA diet contained 7% menhaden oil (a type of fish oil) in place of soybean oil. Our rationale for these dose selections is detailed elsewhere [18,19]. Briefly, we considered this an excess ω-3 FA diet because it had a ω-3/ω-6 ratio that was 100 times what is adequate for pregnant and lactating rats and because our diet of 7% fish oil is consistent with other studies on "excess ω-3 FA" reporting adverse developmental effects (see Introduction). All diets were formulated according to AIN-93G standards which have determined that the ω-3/ω-6 ratio ~ 0.14 and 7% oil composition is ideal for pregnant and lactating female rats [50]. Fish oil was selected for the Excess diet because of its use in clinical studies (see Introduction). The soybean and safflower oils in the Control and Deficient diets were selected because they are commonly consumed by humans and used in animal studies. We used the naturally occurring fatty acid profiles of the fish, soybean and safflower oils; nothing was artificially altered. The diets were prepared by Dyets Inc (Bethlehem, PA). All three diets contained tertiary-butylhydroquinone (TBHQ) because this preservative prevents oxidation [23,24,55]. Diets were stored at refrigeration temperatures and fresh diet was provided twice weekly to further protect against oxidation. Each diet provided 3.96 kcal/g. Detailed composition of each diet was recently published in this journal [18,19].

Dams had free access to food and water. Food consumption was assessed twice weekly when the old food was discarded and replaced by fresh diet. Dams were weighed on these days as well. Animals were housed at ~53% relative humidity and at ~22 °C room temperature. Within 24 h after delivery, designated as postnatal day one (PND 1), litters were counted, weighed and reduced to 8 pups per litter, consisting of 4 male and 4 female pups when possible. The remaining pups were euthanized by CO_2 exposure and decapitated to ensure death. The retained pups were weaned on PND 21 and kept on their respective experimental diets until the day of ABR testing on PND 24. After PND 24, all offspring channeled into our ABR study were switched to a standard rodent diet (5001 Rodent Diet, PMI Nutrition International).

2.2. ABR procedure

When possible, one male and one female pup/litter were randomly selected for testing in the current study. The other littermates were channeled into a study that analyzed their fatty acid tissue composition [35]. Using male/female littermate pairs allowed assessment of sex-dependent differences and controlled for within-litter effects by limiting the number of pups tested from any one litter. Of the 23 Control litters, 21 litters had both a male and female offspring that were ABR-tested whereas one litter had just one male and one litter had just one female offspring that were tested for a total of n=44 offspring. Of the 31 Deficient litters, 30 litters had both a male and female offspring whereas one litter had just one female offspring for a total of n=61 offspring. Of the 22 Excess litters, 21 litters had both a male and female whereas one litter had just one male offspring for a total of n=43 offspring. Rat offspring were initially tested on PND 24 and those test results were previously reported [18,19]. These same offspring were subsequently retested as young adults and those results are reported in this article. The animals were fully mature young adults, aged 167–178 days (95% confidence interval) at the time of retesting. The rat ABR is fully mature and stable by approximately 70–100 days of age [20] and age-related hearing loss (presbycusis) usually does not occur in rats until about 17 months [15].

Our ABR procedure is detailed elsewhere [16,18]. Prior to ABR recording, each animal was given 100 mg/kg of the anesthetic ketamine (i.p.). Ketamine influences ABR latencies and amplitudes, but the effects are minor and the ABR quality is excellent [17]. Rectal temperature was monitored because temperature can influence the