The Effect of Low Omega-3/Omega-6 Ratio on Auditory Nerve Conduction in Rat Pups

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Abstract- The biological effects of omega-3 and omega-6 fatty acids are determined by their mutual interactions. This interaction extremely affects various functions. Lower consumption of omega-3 during gestation leads to various disorders, even in hearing. We aimed to assess the effect of low omega-3/omega-6 ratios on auditory nerve conduction. In this experimental study, the auditory brainstem response test was performed on a 24-day-old rat (n=14). The rats were divided into case (low omega-3/omega-6 ratio during gestation and lactation) and control groups. Variables such as P1, P3, and P4 absolute latency period, interpeak (P3-P4, P1-P3 and P1-P4), and P4/P1 amplitude ratio were measured. We found an increased P4 omega-3/omega-6 ratio in the group with a low omega-3/omega-6 ratio (P<0.01). No significant difference was observed in the P1 and P3 absolute latency period between the studied groups (P>0.05). Also, no significant difference was observed between the groups with respect to the P1-P3 interpeak latency (IPL) periods (P>0.05); while the P1-P4 and P3-P4 IPLs were significantly increased in the group with a low omega-3/omega-6 ratio (P<0.05). The P4/P1 amplitude ratio significantly decreased in the group with a low omega-3/omega-6 ratio (P<0.05). Results confirmed the negative effects of low omega-3/omega-6 ratio on the auditory system and hearing.

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Keywords: Omega-3/omega-6 ratio; Auditory nerve conduction; Rat pups

Introduction

Omega-3 and omega-6 are unsaturated compound fatty acids, which cannot be synthesized by the human body and are received through dietary intakes. Omega-3 fatty acids play an essential role in metabolism. Alpha-Linolenic acid (ALA) is the only essential fatty acid that the body cannot synthesize; however, if included in the diet it can form eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) that are considered as long-chain omega-3 fatty acids, that the body can synthesize (1,2).

Linolenic acid (LA), as the only essential fatty acid of omega-6 fatty acids, is used in the biosynthesis of long-chain arachidonic fatty acids. However, there is still a competitive effect between omega-3 and omega-6 for saturation reducing enzymes, and high consumption of LA interferes with the lengthening of the ALA chains (3). The biological effects of omega-3 and omega-6 fatty acids are evident through their mutual interaction. This interaction affects various functions (4) including eicosanoid and lipoxin formation, producing lipid particles for cell signaling, and activating DNA transcription factors (2). Omega-3 fatty acids because of their lipid structure and myelination ability are key compounds in nerve conduction processes, the plasma membrane, and visual and cerebral development (5,6). Omega-3 is a ligand for the retinoid receptor in nerve tissue. Moreover, it can activate signaling pathways that are crucial for regulating gene expression (7).

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Considering the current change in eating habits and the lower consumption of omega-3 fatty acids that is in turn accompanied by an increase in the omega-6/omega-3 ratio, the prevalence of various diseases has increased (8). Some studies have shown that reduced consumption of omega-3 during gestation can increase the risk of cerebral palsy, attention disorders, hyperactivity, memory disorders, lower IQ, and lower birth weight in newborns (9). Moreover, some researchers have found that the neural complications of omega-3 deficiency also affect the auditory system; however, these studies have mostly conducted in animal. The results and applications of such studies, especially those focusing on auditory brainstem responses (ABRs), have been extended to the whole nervous system (10,11).

ABRs could be used for assessing the development of the cochlea, auditory nerve, and brain stem regarding the effective role of fatty acids, especially omega-3 in creating cell membranes, myelinization of nerves, and metabolism (12). Deficits and high levels of dietary intake of omega-3 during gestation and lactation lead to prolonged neural transmission times increased hearing threshold, delayed acoustic reflex, and disorders of myelination of the brain in rat models (4). Bourre and colleagues had assessed the effect of ALA deficit on ABRs, and found that the amplitude and ABR wave latency periods progressively increased in the group with low omega-3 diet compared with those on a diet rich in omega-3 (13). Church and *et al.*, also found similar results (14).

Considering the importance of different omega-3/omega-6 ratios on various functions, we aimed to assess the effect of omega-3/omega-6 ratio on auditory nerve conduction that has not been well studied yet.

Materials and Methods

The protocol of this experimental study was approved by the Ethics Committee of Tehran University of Medical Sciences (Code: 91-01-32-17279), Tehran, Iran. All related experiments were performed according to the University's laws and regulations of working with laboratory animals.

Animals/diets

A total of 14 female Wistar rats, 10 weeks of age, weighing 180-250 g were purchased from Pasteur Institute, Iran. The rats were housed at a temperature of 22-24°C, 50% humidity, and 12-hour light/dark cycles for one week to adapt to the environment. Food and water were provided at libitum. Then, the female rats were randomly put in separate cages with some male rats with a 2/1 ratio. After mating and confirmation of pregnancy by vaginal plug (showing the day 0 of pregnancy), the female rats were separated from the male rats and were put and kept in special cages. At this stage, the dams were randomly divided into two groups. The control group received a diet conforming to the American Institute of Nutrition 93-Growth: AIN93G and the case group (n-3 polyunsaturated fatty acid [PUFA] deficiency) had omega-3 intake deficiency based on the same diet. The details of the received diet are shown in table 1.

All the animals received 3.97 kcal/g from the diets. The dams in each group received the specified diets during gestation and lactation. The dietary compounds were qualitatively controlled and analyzed by the National Nutrition and Food Technology Research Institute. Soybean oil (Jahan Iran Company) with an omega-3/omega-6 ratio of 0.012 and a combination of safflower and almond oil with an omega-3/omega-6 ratio of 0.002 was used for the control and case groups, respectively. Table 2 shows the fatty acid composition of the two oils used in the study.

Table 1. Composition of the diets (g/kg) received by each group

Nutritional Substance	Control Group	Case group (n-3 PUFA Deficient)
Casein	200	200
Cornstarch	397.486	397.486
Dextrinized cornstarch	132	132
Sucrose	100	100
Cellulose	50	50
peanut and safflower oils	-	70
Soybean oil	70	-
Vitamin mix	10	10
Mineral mix	35	35
L-cystine	3	3
Choline bitartrate	2.5	2.5
Tert-Butylhydroquinone	0.014	0.014
Calories (kcal/g)	3.96 kcal/g.	3.96 kcal/g.

ABR procedure

Two male pups in each delivery were selected using the simple randomization method. The ABR procedure was performed single-blindly on 14 pups in each group on postnatal day 24. According to previous studies, ABR is well-developed at this time in rats (15). Before recording the ABRs, anesthesia was induced using injections of ketamine (40 mg/kg weight) and xylene (10 mg/kg weight). Ketamine can influence the rodent ABR

latencies and/or amplitudes, but the effects are minor, and more importantly the thresholds are not altered, and ABR quality is excellent (14). Since the temperature can affect ABR results (7), normothermia was maintained using a heating pad.

Table 2. Fatty acid composition of the two oils used in the study (% of total fatty acids)

Fatty acid	Soybean oil	Peanut + Safflower oils
12:0	-	-
14:0	0.07	0.03
16:0	10.97	6.8
17:0	-	-
18:0	4.17	2.2
20:0	0.35	-
22:0	0.36	-
24:0	-	-
16:1(n-7)	-	-
17:1(n-8)	-	-
18:1(n-9)	25.85	67.8
22:1(n-9)	0.01	-
17:4	-	-
18:2(n-6)	51.40	22.4
20:3(n-6)	-	-
20:4(n-6)	-	-
18:3(n-3)	6.34	0.06
18:4(n-3)	-	-
20:5(n-3)	-	-
22:5(n-3)	-	-
22:6(n-3)	-	-
n3/n6	0.12	0.002

The procedure was performed using the Biologic device (Natus, USA). First, a 60×30×30 acoustic box was made. The calibration of the received acoustic intensity level at all studied frequencies in the animal's ear was measured using a 1.3-octave band filter and sound level meter (Norsonic, Norway) on Impulse and Peak modes, and the evoked response recording device was calibrated based on the shown sound pressure level. During the study, the non-inverting, inverting, and the ground electrode were placed on the vertex, behind the experimented ear, and behind the other ear, respectively.

The impedance of the electrodes was evaluated for beginning of the procedure, and it was less than 2 kilo-ohms on average for all the samples, which conformed to the standard amounts. Moreover, the inter-electrode impedance was also less than two kilo-ohms. The click stimulus was presented using a high-frequency loudspeaker with a frequency of up to 20 kHz with an intensity level of 100 peak-equivalent sound pressure level or peSPL (duration=100 μs, polarity=refraction, repetition rate=11.1/s). A time window of 10.44 ms and a 100-3000 Hz digital filter was considered. The noise rejection level in this study was 47.3 dB SPL.

ABR was performed with the click stimulation in diagnostic goals and threshold and frequency response were not measured in this method.

The ABR consists of four positive peaks (P1-P4), 6 ms after presenting the acoustic stimulus (16). These peaks mainly represent the activities of the auditory nerve (P1), cochlear nucleus (P2), the superior olivary complex (P3), and the lateral lemniscus and/or inferior colliculus (P4) (17,18).

The absolute latency periods of P1, P3, and P4 depict the neural transmission time along the nerve and brain-stem. In this study, we evaluated the absolute latency periods of P3-P4, P1-P3, and P1-P4. The interpeak latency period P1-P3 (P1-P3 IPL) probably measures the neural transmission from the auditory nerve to the superior olivary complex. The P1-P4 IPL and the P3-P4 IPL measure the neural transmission time in the brain-stem beyond the auditory nerve and the upper parts of the brain-stem, respectively (18). The P4/P1amplitude ratio was measured as the third variable.

Data were analyzed using SPSS software, version 11(SPSS Inc, Chicago, Ill, USA). The Mann-Whitney test was used for comparing the variables between the groups. $P<0.05$ was considered as statistically significant.

Results

Table 3 shows the mean ± SD absolute latency period. As shown, no significant difference was seen in the absolute latency periods of P1 and P3 between the studied groups ($P>0.05$). However, the absolute latency period of P4 differed significantly between the groups ($P<0.01$) and an increased P4 absolute latency period was observed in the case group.

Table 3. ABR Absolute latency (ms) as a function of Diet Group (mean ± SD)

Absolute latency	Control group	Case group	*P*.value
P1	1.43±0.11	1.43±0.05	0.45
P3	3.14±0.16	3.18±0.15	0.45
P4	4.21±0.07	4.49±0.33	0.006

Table 4. ABR Interpeak latency (ms) as a function of Diet Group (mean ± SD)

Interpeak latency	Control group	Case group	*P*.value
P1-P3	1.71±0.21	1.76±0.16	0.94
P3-P4	1.06±0.13	1.30±0.16	0.006
P1-P4	2.85±0.35	3.05±0.35	0.044

The studied groups did not differ significantly with respect to the P1-P3 ILP ($P>0.05$), while P1-P4 and P3-

P4 ILP had significantly increased in the case group (P<0.05) indicating the effect of a diet lacking omega-3 fatty acids on auditory nerve conduction (Table 4). Authors found that the mean P4/P1 amplitude ratio significantly declined in the case group compared with the control groups and this variable changed as a function of the dietary regimen (P<0.05).

Discussion

Current study indicated that reduced omega-3/omega-6 ratios during pregnancy and lactation increased the neural conduction period in newborn rats. The ABR latency periods assess the transmission speed in the auditory system from the cochlea to the inferior colliculus located in upper sections of the brain-stem. It is a reflection of the extent of myelinization in the central nervous system in premature or term neonates (4). The effects of dietary regimens were only significant in the absolute latency period of P4 while no significant increase was observed in the P3 and P1 latency periods. Increased ABR absolute latency periods could indicate weak neural myelination or synoptic disorders in the ABR.

Present results are consistent with those obtained by Church and colleagues in 2008, 2009, and 2010, although the rats in the current study had mild omega-3 deficiency (omega-3/omega-6 ration=0.002 *vs.* 0 in the mentioned studies). The mentioned studies also showed a significantly increased absolute latency period in P4 but not in P3 and P1 (14,18,19). Bourre and co-workers assessed the effect of ALA deficiency on ABRs in different age groups and found no significant difference in P1 latency periods. However, P3 latency periods had a higher progressive rise in the omega-3 deficient group compared with the normal group (13). The results derived from the mentioned studies could be due to the lack of valid research on the difference in wave origins and forms in previous years. Current studies show that P3 cannot be a suitable basis for measuring ABR parameters (20).

Studies indicate that omega-3 and omega-6 levels have significant effects on the dopamine levels in the frontal cortex, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin, 5-hydroxyindolacetic acid, inferior colliculus, and striatum, leading to their lower concentration in animals on an diet with inadequate amounts of omega-3. Inadequate amounts of dietary omega-3 lead to reduced dopamine levels and 5-hydroxyindolacetic acid in the lateral lemniscus and inferior colliculus, and regions related to visual and auditory information processing. La Presa *et al.,* (2000) study reported significant difference between animals and neonates that have different dietary intakes of omega-3.Moreover, omega-3 not only affects myelination, but also alters the metabolism of neural transmitters and, therefore, can increase latency periods of all peaks, especially P4 (21).

Present study showed increased P1-P4 and P3-P4 ILPs as a result of neural transmission delay in paths between the neural conductors of the mentioned waves that is in turn caused by lower omega-3/omega-6 rations. However, the difference was not significant between the studied groups with respect to P1-P3 ILP. Other studies also showed increased P1-P4 ILP in the group with omega-3 deficiency. Also, it should be noted that the researchers only used this index for assessing neural transmission time along the brainstem (19,22). Previous related studies have not assessed P3-P4 ILP as a diagnostic parameter (16). We studied this parameter as part of the effective path in creating P1-P4 ILP to determine the origin and path of neural transmission disorders in the omega-3 deficient group. Thus, it might be inferred that increased P3-P4 ILP affects P1-P4 ILP to some extent. The above mentioned results confirm disorders in the anatomical regions related to P3 to P4 peaks or, in other words, the upper parts of the brain-stem.

We found a significant difference between the studied groups with respect the P4/P1 amplitude ratio, which had decreased in the case group. ABR amplitude depends on the number of neurons that are simultaneously drained. In fact, ABR amplitudes reflect the number of neural triggers that in turn depend on neural simultaneity (4,11). The above mentioned results could be explained by the susceptibility of neural transmitter, especially in the inferior colliculus as a probable origin for P4, which is influenced by the dietary regimen (23). However, since the mentioned index had not been assessed in previous studies, we did not have a sample for comparison. It seems that tissue assessments accompanied by electro-physiological experiments could confirm the obtained results of the mentioned study, which could be considered in future studies.

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