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RESEARCH ARTICLE

Hearing estimated threshold recovery after administering fish oil in sucking periods in n-3 fatty acid-deficient rat pups

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Abstract

Background and Aim: Omega 3 of essential fatty acids, especially in fish oil form plays an important role in structural, functional, and biological aspects in body. Omega 3 deficiency can have devastating effects on the nervous system and auditory. This study aimed to evaluate auditory threshold in n-3 fatty acid-deficient rat pups following supplementation of fish oil consumption during the suckling period.

Methods: One sources of omega 3 fatty acid were fed to rat pups of n-3 polyunsaturated fatty acids (PUFA)-deficient dams to compare auditory thresholds in 2, 4, 8, 12 and 16 kHz by auditory brainstem response (ABR) among this group, those with defects with placebo, and control groups. The pups' in supplement group were orally fed 5 mol/g weight of fish oil between the ages of 5 and 21 days.

Results: Results showed significant differences in auditory threshold estimation in 2,4,8,12 and 16 kHz in ABR between study groups. The group with n-3 PUFA deficient with placebo showed a significant increase of the parameters indicated as $p<0.05$. Moreover a lower threshold (better) in n-3 PUFA deficient with the fish oil in comparison with n-3 PUFA deficient with placebo ($p<0.05$).

Conclusion: The result of study showed effect of omega 3 deficiency on auditory during pregnancy and lactation period and compensation neural auditory threshold in n-3 fatty acid-deficient rat pups following a supplementation of fish oil consumption during the suckling period**.**

Keyword: Fish oil, auditory thresholds, n-3 fatty acid-deficiency, rat pup, auditory brainstem response

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Introduction

Fish oil contains omega 3 fatty acid. Omega 3 (n-3 fatty acid) are member of polyunsaturated fatty acids (PUFA). This fatty acid with other long chain polyunsaturated fatty acids named omega 6 have an important role in body [1]. Currently, due to a change in common dietary in the world, defect of omega 3 consumption and increasing omega 6/omega 3 ratio can be seen; as a result the prevalence of various diseases by affecting on different organ in body has increased.Thus the modification of diet pattern and a balance between omega 3 and omega 6 are considered [2]. The role of omega 3 investigated in fetal development, birth and growth of newborn, brain and nervous system development, cognition, memory, visual,

decreased brain myelination and cause abnormal neurobehavioral function, auditory and lipid composition in other organs [2-4].

Studies in neural field expanded to auditory. However, more studies on this system have been done on animal. Many studies used an ABR test that is an important tool for measuring sensory development of the central auditory nervous system. ABR is a benefit tool for detecting the influence of diet on development of sensory and neural systems [5]. There is also a strong correlation have reported between the level of fatty acids, especially docosahexaenoic acid (DHA; 22: 6) and myelination of auditory nerve in some studies [6]. Results of studies reported defect of auditory system with n-3 deficiency as well as a delay in the acoustic startle reflex, poor auditory acuity, and impaired auditory nerve conduction with excess omega 3 fatty acid consumption [5,7-9]. In spite of all researches in auditory field, no studies have investigated a way to reduce or prevent adverse effect of n-3 fatty acid-deficient consumption during pregnancy and lactation on hearing acuity in rat pups. In recent years, studies on animal models to repletion of n-3 fatty acid-deficient dams with a-linolenic acid in brain and liver were done. The results of these studies showed improved levels of DHA in these tissues [10, 11].

This prompted us to undertake the current investigation, where we examined the effect of n-3 PUFA deficient nutritional status on auditory acuity and we investigated the effect of fish oil (omega3) supplementation during the suckling period on auditory estimated thresholds (2-16 kHz) in n-3 fatty acid-deficient rat pups by comparing the results of ABR thresholds between n-3 PUFA deficiency with placebo and n-3 PUFA deficiency who were supplied with fish oil. Perhaps the results of study would suggest an important way in improving auditory acuity in n-3 PUFA deficiency through the supplementation of fish oil in the suckling period.

Methods

All animal procedures were performed according to the Regulations for Animal Experiments in Tehran University of Medical Sciences (October,2005) and with ethical approval (project No 91-01-32-17279) in ethical committee.

Animal, diets, and supplementation

In this experimental study, 12 female wistar rats (Approximate weight: 180-250 g) were purchased from Pasteur Institute (Tehran, Iran). All animals were housed at Temperature of 22 - 24 ° C and kept for a week to assent with condition. They were mated with male wistar rats (2 to 1 ratio). The presence of a sperm plug was designated as gestational day one. Randomly, 12 females were assigned to one of the two diet conditions starting from day one of pregnancy. The two diets were formulated according to American Institute of Nutrition 93-Growth (AIN93G) Standard as a control diet and the n-3 PUFA-deficient diet by Dyets Inc (Bethlehem, PA, USA) and Damloran razak Inc (Boroujerd, Iran). The detailed composition of each diet is given in Table 1.

All rats received same energy (3.97 kcal/g) from different diets. Both groups of rats used their own diet during pregnancy and lactation. These materials in diets were analyzed by National Nutrition and Food Technology Research Institute.

The control diet was made with soybean oil, while the n-3 deficient diet contained a combination of peanut and safflower oils in place of soybean oil. The n3/n6 ratio in soybean oil in control group was 0.012 and 0.002 for n-3 PUFA-deficient oil.

Menhaden oil supplement (Dyets Inc, Bethlehem, PA, USA) was used as sources of n-3 LCPUFA and emulsified in 1% sodium carboxymethyl cellulose. Fatty acid compositions of three oils are given in Table 2.

Da ms were fed the experimental diets through the gestation and lactation periods. After pregnancy period and gestation (21days), Male pups in n-3 PUFA-deficient diet group derived two groups. Pups in one of these groups was orally administered fatty acid supplements at 5 mol /g of body weight to pups from 5 to 21 days

Table 1. Composition of the diets based on AIN93G

Nutritional Substance	Control	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.

of age (n=6). Also other male pups of n-3 PUFA-deficient diet (n=6) and the control diet were administered the placebo (n=6). The oil or placebo administration ended on the weaning day. After weaning, pups were fed the same diet as their dams for a 1-week without oil or placebo administration. After 1-week period without supplement (28 days of age), rat pups were prepared for ABR test (Figure 1).

ABR Procedure

Prior to ABR recording, each animal was given 40 mg/kg of the anesthetic ketamine and 10 mg/kg of xylazine. Temperature was monitored because temperature can influence the ABR [8]. A heating pad was used to maintain normothermia.

For ABR test, firstly, a sound box (30 ×60× 30 cm) was made. ABR testing was performed blind and rat pups from each delivery randomly selected and categorized in each group.

Unlike previous methods, the custom tone burst stimuli in 2000, 4000, 8000, 12000 and 16000 Hz were delivered through high frequency loudspeaker (HD250) with flat frequency response plot to 20 kHz positioned directly in front of the animal in 100 dBSPL (rise/fall = 0.5 ms, plateu =10 ms, polarity=alternate, repetition rate=23.1/s). Calibration intensity level in sound box performed with Norosonic sound level (Norsonic, Norway) (1/3 octave, impulse and peak state). The ABR was differentially recorded between two subcutaneous platinum E-2 needle electrodes. The active (noninverting) electrode was inserted at the vertex, the reference (inverting) electrode below the left ear, and the ground electrode below the right ear. Evoked potentials were collected by a Biologic Navigator (Natus, USA) and amplified by a factor of 300,000 times with a digital bandpass of 100-3000 Hz. Electrode impedances ranged from 0-5 kΩ. At least 256 responses were averaged. The amplified signals were averaged with positivity displayed upwards and traces stored on computer disk for later analysis.

Table 2. Fatty acid composition of the 3 oils used in the study (% of total fatty acids)

Fatty acids	Soybean oil	peanut + safflower oils	Menhaden oil supplement
12:0	-	-	0.11
14:0	0.07	0.03	7.96
16:0	10.97	6.8	19.88
17:0	-	-	1.17
18:0	4.17	2.2	1.99
20:0	0.35	-	0.61
22:0	0.36	-	0.32
24:0	-	-	0.20
16:1 (n-7)	-	-	10.69
17:1 (n-8)	-	-	2.24
18:1 (n-9)	25.85	67.8	16.38
22:1 (n-9)	0.01	-	0.23
17:4	-	-	3.91
18:2 (n-6)	51.40	22.4	2.26
20:3 (n-6)	-	-	0.38
20:4 (n-6)	-	-	0.20
18:3 (n-3)	6.34	0.06	2.04
18:4 (n-3)	-	-	3.68
20:5 (n-3)	-	-	1.90
22:5 (n-3)	-	-	2.53
22:6 (n-3)	-	-	14.68
n3/n6	0.12	0.002	11.04

The analysis epoch was 10.24 ms. An artifact rejection system eliminated individual responses if they contained intensity exceeding 47.3 dB SPL. ABRs were gathered to a range of stimulus intensities starting at 100 dB peak-equivalent sound pressure level (peSPL), and then decreased in steps of 10 dB as the ABR threshold was reached. At least two ABR traces were collected at each near threshold intensity level. Threshold was defined as the lowest Intensity to elicit a reliably scored ABR component. Threshold measurements were performed with P2 wave. All data are expressed as the means standard deviation (SD). Due to limited size of sample, Kruskal Wallis nonparametric test was used to determine whether the values of variables in n-3 PUFA deficient with the fish oil supplement, n-3 PUFA deficient with placebo, and control groups differed from each other. Mann-Whitney test was used for comparisons between pairs of groups. The criterion for statistical significance

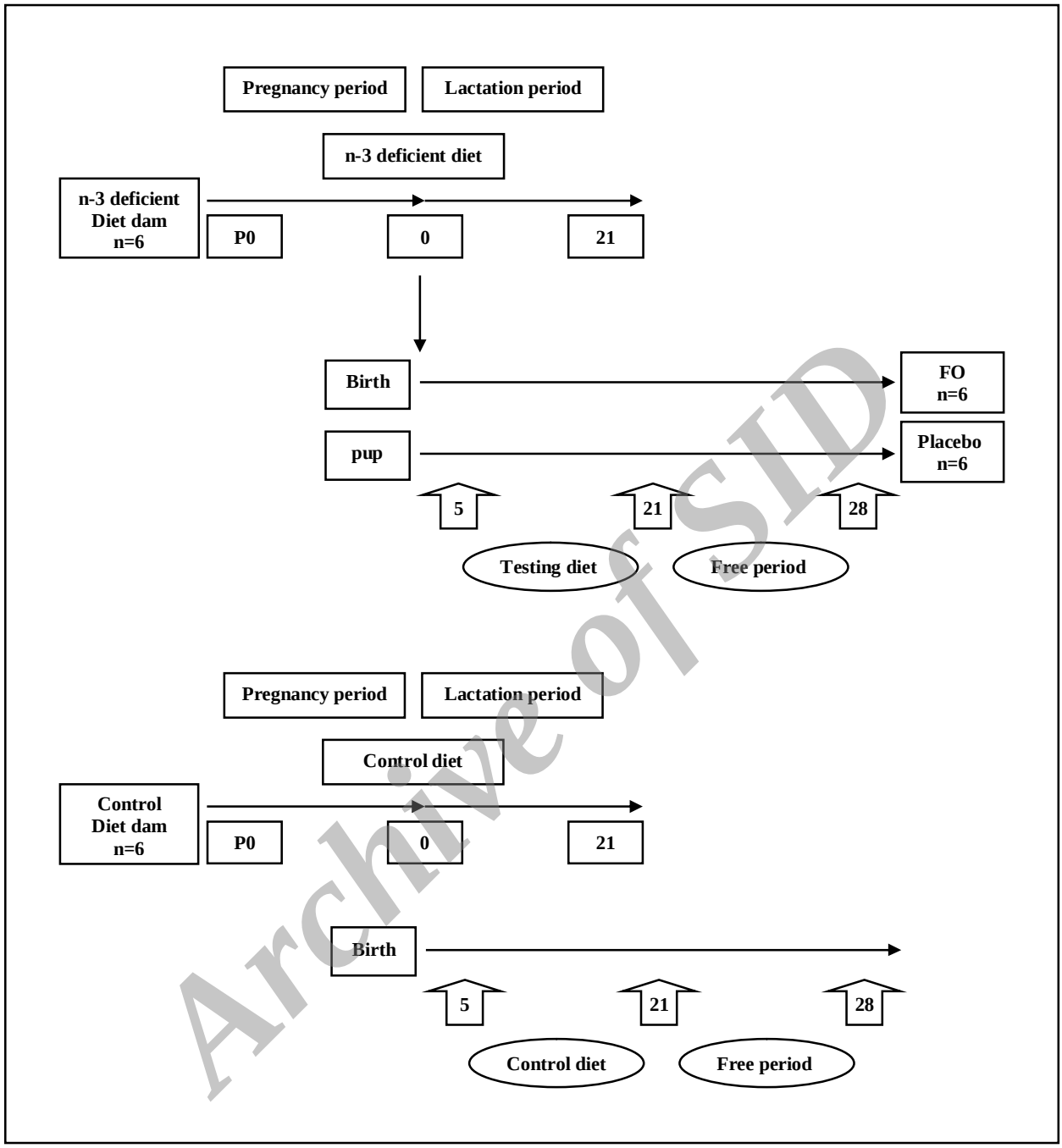

Figure 1. Block diagram of experimental procedure

was $p \leq 0.05$. SPSS version 20 was used for all statistical calculations.

Results

ABR thresholds in all frequencies are given in Table 3. ABR thresholds estimated with Kruskal Wallis nonparametric test in 2, 4, 8, 12 and 16 kHz between the control group, n-3 PUFA deficient with placebo, n-3 PUFA deficient with the fish oil supplement. The results showed

Table 3. Mean and SD of ABR thresholds (dB) as a function of diet group in each frequency (kHz)

frequency (kHz)	Control	n-3 PUFA deficient with placebo	n-3 PUFA deficient with FO	p	p comparison between control and n-3 PUFA deficient with placebo group	p comparison between n-3 PUFA deficient with placebo and n-3 PUFA deficient with fish oil supplement group	p comparison between control and n-3 PUFA deficient with fish oil supplement group
2	26.6 (2.58)	37.5 (2.73)[ab]	24.16 (8.01)	0.002	0.003	0.003	0.83
4	21.6 (4.08)	25.83 (2.04)	15 (5.47)	0.003	0.045	0.003	0.037
8	19.16 (3.76)	23.33 (2.58)	14.16 (4.91)	0.007	0.05	0.004	0.067
12	19.16 (2.04)	26.66 (2.58)	15.83 (5.8)	0.001	0.002	0.003	0.21
16	18.33 (2.58)	27.50 (4.18)	13.33 (6.8)	0.002	0.003	0.003	0.16

significant differences in auditory thresholds estimation in above frequencies between studied groups ($p<0.05$). Although to accept or reject hypothesis, comparison was performed between pairs of groups. Statistic analysis showed the most significant increase in ABR estimated thresholds (the worst) in n-3 PUFA deficient with placebo compared with control and n-3 PUFA deficient with fish oil supplement groups ($p<0.05$). Specifically, ABR estimated thresholds were the highest (worst) for the 2 kHz and got lower (better) in 16 kHz. Significant differences were found in ABR thresholds in all frequencies between n-3 PUFA deficient with fish oil supplement and n-3 PUFA deficient with placebo groups ($p<0.05$)(Figure 2). Lower ABR thresholds (better) in n-3 PUFA deficient with fish oil supplement groups were seen in comparison with n-3 PUFA deficient with placebo ($p\leq0.05$). No significant differences ($p>0.05$) were found in ABR estimated thresholds between control and n-3 PUFA deficient with fish oil supplement groups except 4 kHz (Table 3).

Discussion

We found that mild deficit of n-3 fatty acid in maternal diet during pregnancy and lactation, caused elevated ABR thresholds in their offspring at 28 days of age. This effect was significant in all frequencies. Although the frequency of 8 kHz was borderline significant. The means of ABR thresholds at different frequencies roughly matched with physiology of auditory in rat as ABR thresholds were the highest (worst) for the 2 kHz tone pip condition and got lower (better) as tone pip frequency increased to 4, 8 and 16 kHz. Elevated ABR thresholds in 2, 4, 8, 12 and 16 kHz are indicative of poorer auditory acuity in n-3 PUFA deficient with placebo group than control group caused by a deficiency of omega-3 intake. This finding was in line with the results of Church et al. who demonstrated that omega 3 deficiency in daily diet has significant effect on ABR thresholds. The results of study showed elevated ABR thresholds in 2, 4 , 8, 12 and 16 kHz [4], and reduced amplitude of P2 to P4. So that P 2 was seen at a higher intensity level. These results of investigation showed ABR thresholds as a function of diet group.

Research in recent years by the same group showed higher auditory thresholds in PUFA deficient group, although it was significantly less than the expected value. The thresholds at 2 kHz were significantly higher than other frequencies [8]. The results of present study were matched with previous studies, however, in our study p value was less.

A balanced ratio of omega 3 and omega 6 fatty acid is an important factor because is required for optimal growth. A relative defect of ω-3 fatty acid would create higher arachidonic acid (AA) concentrations in blood, brain and other tissues through competitive displacement that could shift the balance of metabolic hormones.

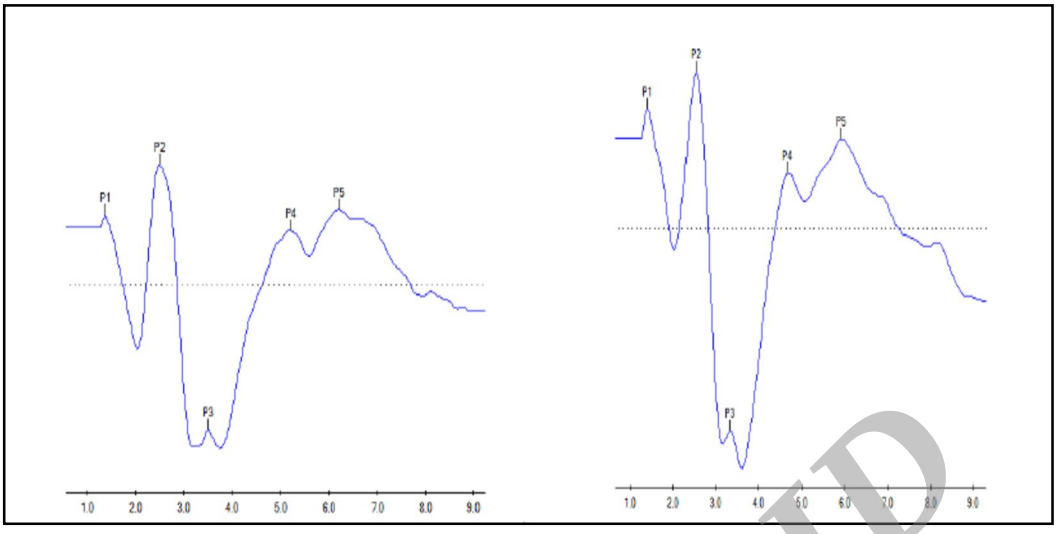

Figure 2. ABR waveforms in n-3 PUFA deficient with placebo group (A) and n-3 PUFA deficient with FO group

In addition, this imbalance could have an adverse effect on neuronal myelination and synaptic growth along brainstem portions of the auditory pathway [4].

Our investigation showed a decrease in ABR thresholds in all frequency after administering fish oil supplements by n-3 fatty acid-deficient in sucking period compared to n-3 PUFA deficient with placebo. However there is no significant difference between mean of ABR thresholds in n-3 PUFA deficient with fish oil supplement and control groups. Although there are few studies in this subject but Kimura et al. examined the effect of DHA-rich microalgal oil (DMO) or fish oil during the suckling period in mildly n-3 fatty acid-deficient Rat Pups. The results of their investigation showed the effectiveness of administering either FO or DMO in the pre-weaning period for improving fatty acid compositions (especially DHA) of brain, heart, kidney and phospholipids in myelin [11]. Sarda et al. (1991) found DHA consumption caused growth of the nervous system especially cochlear nucleus and other structures in auditory pathway. Thus we would expect an improvement in the response of the auditory after supplementation [12]. Gopinath et al. preformed a research about consumption of omega-3 fatty acids and fish and risk of age-related hearing loss with pure tone audiometry. The results showed reverse coloration between high intake of fish (omega-3) per week and hearing loss [13]. Salvati and et al. (1996) reported rats that received fish oil supplements showed lower levels of myelin basic protein, reduction of "-3'-cyclic nucleotide 3"-phosphodiesterase (CNPase) activity (indicator of myelination), and an increase in startle reflex. They believe that the decrease in enzyme activity is due to the instability in the structure of myelin [14].Therefore, determining the appropriate amount of supplements for neural repair and a decrease in startle reflex (hearing estimation) may be necessary and we study this subject in present research. In this study, we used the placebo to reduce interference caused by the stress induced by oral administration. Also the temperature was fixed and we tried to get results without bias. Due to limitations on time and resources, we were not able to do anatomical or behavioral studies. It is suggested that a study will be conducted with larger sample size and histological evaluation.

Conclusion

The results of present study showed positive

effect of omega 3 deficiency on auditory acuity in pregnancy and lactation period and compensation auditory estimated thresholds in n-3 fatty acid-deficient rat pups following the supplementation of fish oil consumption during the suckling period. The results of this study can provide the necessary information to prevent auditory damage caused by a lack of omega 3 consumption.

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