Title: Association of Audiometric Measures with plasma long chain polyunsaturated fatty acids in a high-fish eating population: The Seychelles Child Development Study

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ABSTRACT

Objectives: To determine if auditory function is associated with current long chain polyunsaturated fatty acids (LCPUFA) concentrations in a cohort of young adults who consume oceanic fish with naturally acquired methylmercury (MeHg). We measured participants plasma LCPUFA concentrations (total n-3, total n-6 and the n-6:n-3 ratio) and looked for an association with Auditory Brain Response (ABR) latencies and Otoacoustic Emissions (OAE) amplitudes.

Design: Auditory function of 534 participants from the Seychelles Child Development Study (SCDS) main cohort was examined at 19 years of age. Tests included standard pure-tone audiometry, tympanometry, ABR and both Click-Evoked OAE (CEOAE) and Distortion-Product OAE (DPOAE). Associations of LCPUFA status, measured at the time of examination, and auditory outcomes were examined using covariate-adjusted linear regression models. All models were adjusted for sex, prenatal and current MeHg exposure and hearing status.

Results: LCPUFA concentrations were similar for both sexes and when comparing participants with normal hearing (90.4%) to those who had a sensorineural hearing loss in one or both ears (9.6%). When looking at a subset of only hearing impaired participants, LCPUFA concentrations

were similar in those participants who had a mild sensorineural hearing loss as compared with participants that had a moderate sensorineural hearing loss. LCPUFA concentrations were not correlated with current hair MeHg. LCPUFA concentrations were statistically significantly associated with only 6 of 174 ABR and OAE endpoints examined. Four of the 6 significant associations were present in only one sex. In female participants as n-6 concentrations increased, the ABR wave I absolute latency increased for a 60 dBnHL 19 click/sec stimulus. For male participants the interwave I-III latencies for a 60 dBnHL 69 clicks/sec stimulus increased as the n-6:n-3 LCPUFA ratio increased and the interwave I-V interval decreased for a 60 dBnHL 39 clicks/sec stimulus as the n-6 concentration increased. For both sexes interwave latencies were prolonged for the III-V interwave interval for an 80 dBnHL 39 clicks/sec as n-3 LCPUFA concentration increased.

As the n-3 LCPUFA concentrations increased, the amplitude of the 6000 Hz DPOAE in the right ear increased for both sexes. As the n-6:n-3 ratio increased, the amplitude of the 1500 Hz DPOAE in the left ear decreased for females. The amplitude of the CEOAE was not associated with n-3, n-6 LCPUFA concentrations or the n-6:n-3 ratio.

Conclusion: There was no evidence to suggest LCPUFA status was associated with hearing acuity, ABR latencies or OAE amplitudes, even though our participants tended to have higher LCPUFA concentrations as compared to individuals consuming a more western diet. No association was observed between LCPUFA status and a participants hearing status (normal hearing or hearing loss). Although we found a few associations between current plasma LCPUFA status and ABR and OAE auditory endpoints examined, no clear pattern exists. Some of these associations would be considered detrimental resulting in prolonged ABR latencies or smaller OAE amplitudes, while others would be considered beneficial resulting in shortened ABR latencies or larger OAE amplitudes.

Abbreviations Used: ABR (Auditory Brainstem Response), BAER (Brainstem Auditory Evoked Response), LCPUFA (Long Chain Polyunsaturated Fatty Acids), OAE (OtoAcoustic Emission), CEOAE (Clicked Evoked OtoAcoustic Emission, DPOAE (Distortion Product OtoAcoustic

Emissions), AA (arachidonic acid), EPA (eicosapentanoic), DHA (docosahexaenoic acid), MeHg (methyl mercury)

Keywords: Long Chain LCPUFA, Auditory Brainstem Responses, Otoacoustic Emission, Fish Consumption, Seychelles Child Development Study

INTRODUCTION

The auditory system is a reliable objective system in which to look for possible beneficial or adverse associations with nutrients and toxicants. Dziorny and colleagues et al. reviewed the literature on neurotoxicity and auditory physiological measures (Dziorny et al., 2013) and found that in experimental models and in humans, gestational exposure to both LCPUFA and MeHg is reported to influence the auditory system. Peripheral end-organ or outer hair cell function can be assessed with evoked otoacoustic emissions (OAE) and behavioral tests of auditory function such as hearing. Central auditory function can be assessed with the Auditory Brainstem Response (ABR) which measures auditory nerve conduction speed. Both breast feeding as well as LCPUFA supplemented formula are reported to improve brain development in infants as measured by ABR (Amin et al., 2000; Yehuda, 2003; Khedr et al., 2004; Unay et al., 2004; Koletzko et al., 2008;) while MeHg exposure is reported to prolong ABR latencies (Inayoshi et al., 1993; Hamada et al., 1982;). In experimental rodent models, diets supplemented with excessive n-3 LCPUFA can result in detrimental auditory outcomes (Church et al., 2007; Church et al., 2009). Church and colleagues (Church et al., 2007) for example, showed that rats fed a diet of excess n-3 LCPUFA had a higher incidence of hearing loss as they aged as well as other sensory and neurologic abnormalities.

Fish and other seafood contain protein, calories and nutrients such as LCPUFA that are essential for development of the central nervous system (Yehuda, 2003; Koletzko et al., 2008). Fish are the primary human source of two specific long-chain polyunsaturated fatty acids (LCPUFA), eicosapentanoic acid (EPA; 22:5, n-3) and docosahexaenoic acid (DHA; 22:6, n-3). EPA, DHA along with arachidonic acid (AA; 20:4, n-6) play key roles in normal development of the central nervous system. DHA accumulates in the brain, especially around synapses, and is thought to

influence neurotransmitter metabolism, ion channel activity, signaling pathways, gene expression, cell signaling and synaptic transmission via production of eicosanoids (Carlson, 2001; Innis, 2003; Innis, 2005; Makrides et al., 2011). There exists considerable data on the benefits of diet supplementation with LCPUFA in adults. Increases in LCPUFA intake via supplementation or ingestion of foods high in LCPUFA are believed to be beneficial in preventing and reducing the sequelae of cardiovascular disease, mental health conditions and detrimental side effects of aging, metabolic syndromes as well as inflammatory and immune disease (Akabas & Deckelbaum, 2006). People are unable to synthesize adequate amounts EPA, DHA and AA from precursor fatty acids. Diets containing high n-3 reduce the n-6:n-3 ratio and consequently increase the production of anti-inflammatory eicosanoids that are considered beneficial (Calder, 2001; Hibbeln et al., 2006; Simopoulos, 2006). A diet high in n-6; however, has been associated with inflammatory conditions (Calder, 2001; Hibbeln et al., 2006).

Ocean fish contain small amounts of MeHg that is naturally present and bioaccumulated from their environment. The amount is usually well below 1 ppm. Studies of low dose exposure to MeHg from fish have shown no consistent pattern of adverse associations. Whether prenatal and or current low level MeHg exposure from consumption of ocean fish is causally related to adverse neurodevelopmental changes is controversial. Some studies have reported no associations of physiological auditory measures with prenatal MeHg exposure (Weihe et al., 2002; Murata et al., 2004; Orlando et al., 2014; Choi & Park, 2017), while others have reported adverse associations with low dose exposure (Grandjean et al., 1997; Murata, Weihe, Araki et al., 1999; Murata, Weihe, Renzoni et al., 1999; Murata et al., 2004). Human studies of the neurotoxic effects of high dosage MeHg exposure (i.e. fish with up to 40 ppm MeHg) however, have been associated with hearing loss and alterations of auditory brainstem response latencies (ABR) (McAlpine & Araki, 1958; Kurland et al., 1960; Nosaka et al., 1970; Fujisaki et al., 1971; Hamada et al., 1982; Inayoshi et al., 1993).

We have previously reported associations of MeHg exposure with auditory measures in this cohort (Orlando et al., 2014). We found no consistent pattern of associations with prenatal exposure, but there were some associations with recent MeHg exposure. Some authors have speculated that the absence of a clear detrimental association of MeHg exposure with outcomes in a fish eating

population has been masked by the beneficial nutrients present in fish (Strain et al., 2008). We determined the association between recent plasma PUFA concentrations (n-3, n-6 and the n-6:n-3 ratio) and hearing acuity as well as two objective and sensitive auditory function measures (ABR latencies and OAE amplitudes) in a population that consumes oceanic fish as their primary source of protein.

METHODS

Subjects

The Seychelles Child Development Study (SCDS) is a longitudinal observational cohort study examining the association of MeHg exposure from consuming fish with child neurodevelopment. The cohort regularly consumes ocean fish and seafood which contains naturally acquired MeHg (Marsh et al., 1995). The SCDS Main cohort consists of 779 mother-infant pairs who were enrolled 1989-1990 (Marsh et al., 1995). They have been evaluated seven times over now the subsequent 19 years. Mothers at enrollment reported consuming fish with meals an average of twelve times per week. Eighty-two participants were excluded for specific reasons including lack of maternal hair for exposure assessment, medical conditions affecting neurodevelopment or withdrawal from the study (Myers et al., 2003). At 19 years of age there were 697 eligible participants (89.5%) of whom 534 (76.6%) participated in the audiometric test battery. The study protocol was reviewed and approved by the Ethics Committee of the Republic of Seychelles and the Research Subject Review Board at the University of Rochester.

Biomarkers

Both MeHg and LCPUFA biomarkers were used as surrogates for the amount of dietary fish consumption. We focused on measurable biomarkers of exposure for increased accuracy as compared to the likely more error prone dietary recall

Blood was drawn from each participant on the day of testing and was stored and transported at minus 80 degrees centigrade for later analysis. Total lipids were extracted and analyzed from plasma samples at the Nutrition Innovation Centre for Food and Health (NICHE) at Ulster University (Folch et al., 1957). A solid phase extraction using an NH2 cartridge system

conditioned with chloroform and followed by a series of solvent elutions was used to isolate the phospholipids. Total n-3 and n-6 PUFA concentrations were determined (van Wijngaarden et al., 2013) and the ratio of n-6 to n-3 was calculated for analysis. Participants also completed Fish Consumption Questionnaire (FCQ) at 19 years of age, but this information was not used as a dietary biomarker in this investigation.

Prenatal MeHg exposure was measured as total Hg (THg) in maternal hair growing during pregnancy as described previously and reported (Cernichiari et al., 1995). Recent mercury exposure was determined by measuring THg in a 1-cm section of the participant's hair closest to the scalp collected at testing. Methylmercury constitutes over 80% of THg in hair (Cernichiari et al., 1995). Measured exposure to lead, polychlorinated biphenyls (PCBs), and pesticides in Seychellois subpopulations is low (Cernichiari et al., 1995; Davidson et al., 1998).

Evaluation Procedures

Three Seychellois nurses were specifically trained in the audiometric testing procedures which were automated and administered in a quiet room at the Child Developmental Centre (CDC) in Victoria. All clinical personnel were blinded to exposures. The participants were seated in a reclined position for ABR testing and seated upright for the pure-tone screening, tympanometry and OAEs.

A complete description of the testing methods is included in our previous paper (Orlando et al, 2014). Audiometric testing included pure-tone screening, tympanometry, Auditory Brainstem Response and distortion product and click evoked otoacoustic emission testing. Standard pure-tone audiometric testing was administered in octaves from 500-8000 Hz (Hertz) (500, 1000, 2000, 4000, and 8000 Hz) using EarTone 3A insert receivers and a calibrated Grasen Stadler (GSI 39) portable audiometer/tympanometer. Tympanometric testing was used to identify participants who had abnormal middle ear function. Participants were evaluated using the autoTymp function on a GSI 39 portable tympanometer with appropriately fitted TS212 tympanometry tips. Those with type B or C tympanograms based on a modification of Jerger's classification were eliminated from analysis (Innis, 2003; Jerger, 1970). ABRs were obtained using 100 µsec click stimuli presented to the right and left ear at 60 and 80 dBnHL (deciBel above normal hearing level), at 19.9, 39.9

and 69.9 clicks/sec. The actual recording was automated and three runs of 1024 clicks were amplified (x 150,000) and filtered (300-3000 Hz) using a Biologic NavPro with insert receivers. Recordings were measured while the subject lay quietly with their eyes closed. The signal-to-noise ratio (SNR) of the cubic difference ($2F_1 – F_2$) Distortion Product OtoAcoustic Emission (DPOAE was measured for the F_2 frequencies at 1000, 2000, 3000, 4000 and 6000 Hz. The SNR of the Click Evoked OtoAcoustic Emission (CEOAE) was measured in 1/3 octave bands from 1000 – 4000 Hz using an Otodynamics Echoport ILO92 USBII clinical OAE analyzer connected to a laptop computer.

All data were saved and electronically transmitted to the University of Rochester where they were analyzed by an audiologist (MSO) blinded to subject identity and exposure status. For the ABR response, two of the three visually closest waveforms at each presentation level, rate and ear, were averaged and analyzed offline. Absolute latencies of waves I, III and V and I' and V' of the averaged waveform were identified and recorded. The recorded absolute latencies were saved to spreadsheet software and interwave I-III, III-V and I-V latencies were calculated.

Statistical Analysis

Associations with blood LCPUFA concentrations and auditory outcomes were examined using covariate-adjusted linear regression models. All models were adjusted for sex, prenatal and recent MeHg exposure and hearing status. These models were specified *a priori* in analysis plans and all associations were tested with two-sided alternatives using a 0.05 significance level.

Each of the ABR endpoints was fit in a separate model. Each model included measurements from both the left and right ears of each subject and included a random subject's effect to account for the correlation between these two measurements. Each of the OAE endpoints was fit with two separate models (one for each ear). Each of the models was first fit with a sex by LCPUFA interaction, separately for n-3 and n-6 (Model 1) or the n-6:n-3 ratio (Model 2). When the interaction was significant, separate associations were tested on males and females. These interactions were removed if not significant and each model was rerun. If a sex by recent MeHg interaction was significant in our previous analysis, that interaction was always included in the

corresponding models (Orlando et al., 2014). Model assumptions were checked using standard methods, including checking for constant variance, nonlinearity, and normally distributed residuals. No transformations were necessary for any covariates or outcomes. Outliers were identified with the absolute value of standardized residuals ≥ 3.

RESULTS

A total of 534 (76.6%) of 697 eligible participated in the 19 year audiometric test battery at a mean age of 19.1 years (range 18.1 to 20.6 years). Of those, six participants were excluded because of abnormal tympanometric findings and 11 had incomplete audiometric data leaving a total of 517 participants for analysis. Not all participants were able to complete all audiometric test procedures and the number of participants included in each analysis therefore varies. Prenatal MeHg exposure and audiometric data were available on 517 participants. Recent MeHg exposure was determined by the mean child hair Hg concentration at time of testing and was available on 481 (90.1%) of the 517 available participants. Plasma LCPUFA analyses were also available for 481 participants. Participants with both of these biomarkers (recent MeHg and LCPUFA) measured and those who completed the audiometric testing procedures left a total of 438 for this analysis.

For the entire cohort, the prenatal MeHg exposure was on average 6.89 ppm as measured by maternal hair concentrations while recent exposure level as measured by participants' hair concentration was on average 10.32 ppm. Although the average prenatal MeHg was similar for both male and female participants, (6.68 ppm for males and 7.07 ppm for females) on average males were found to have significantly higher recent MeHg exposure at 12.60 ppm as compared to females 8.67 ppm, $p< 0.0001$. Prenatal and recent MeHg exposures measured at 19 years of age were not associated with the presence of a sensorineural hearing loss or the severity of a hearing loss (mild or moderate). Detailed MeHg exposures as compared to prenatal and recent MeHg exposures as a function of sex and hearing sensitivity are published elsewhere (Orlando et al., 2014).

Recent LCPUFA concentrations were not correlated to recent MeHg exposure in this population. Those individuals who had very high Hg levels in their hair had a wide range of plasma LCPUFA concentrations in their blood (n-3 $r = -0.080$, $p=0.092$; n-6 $r = 0.021$, $p=0.646$).

The means and standard deviations for the ABR absolute wave I, III and V latencies are shown in Table 1 as a function of presentation level, presentation rate, sex, and ear. Figure 1 shows ABR wave I, III and V latencies as a function of presentation level, presentation rate, and sex collapsed across ears. Overall, the ABR and OAE responses demonstrated the expected signal characteristics. As the rate of the ABR click rate increased from 19 to 69 clicks/sec or as the ABR stimulus presentation level decreased from 80 to 60 dBnHL, the number of identifiable response peaks decreased ($p<0.001$) and the absolute and interwave latencies increased for both sexes ($p<0.001$). As expected, the earlier response peaks, Wave I and III, became more difficult to detect as the stimulus rate increased and the stimulus intensity decreased. Wave V was also affected but only minimally. Female participants had a greater number of identifiable response peaks (waves I, II and V) ($p<0.001$) and had shorter absolute and interwave latencies for all waves (I, III and V and I-III, I-V and III-V) as compared to male participants ($p<0.001$).

Means and standard deviations for the DPOAEs and CEOAEs as a function of frequency, ear and sex are shown in Table 2. The amplitude of the OAE increased as the frequency of the emission increased independent of sex from 1000-4000 Hz for DPOAEs and from 1000-3000 Hz for CEOAEs. Female participants had larger DPOAE amplitudes at 6000 Hz and larger CEOAE amplitudes from 1500-4000 Hz than males. We previously reported that both male and female participants had larger OAE amplitudes for both CEOAEs ($p=0.004$) and DPOAEs ($p=0.003$) in the right ear as compared to the left (Orlando et al., 2014).

Table 3 shows the mean and standard deviation of the plasma LCPUFA concentration (n-3, n-6 and n-6:n-3 ratio) as a function of sex, hearing status (normal hearing or hearing loss) and degree of hearing loss (mild versus moderate sensorineural hearing loss). LCPUFA concentrations were similar for both sexes also when comparing the participants with normal hearing (90.4%) to participants who had a sensorineural hearing loss in one or both ears (9.6%). Additionally, when looking at a subset of only hearing impaired participants, PUFA concentrations were similar in those participants who had a mild sensorineural hearing loss as compared with participants that had a moderate sensorineural hearing loss. No participants had greater than a moderate sensorineural hearing loss in either ear.

Plasma LCPUFA was statistically significantly associated in 6 of 174 ABR and OAE endpoints (Tables 4 and 5 respectively). The associations did not change after accounting for confounders such as sex and ABR click presentation rate and OAE ear and sex. Four of the 6 significant associations were present in only one sex. In female participants as recent n-6 LCPUFA concentrations increased, the ABR wave I absolute latency increased for a 60 dBnHL 19 click/sec stimulus. For male participants the interwave I-III latencies for a 60 dBnHL 69 clicks/sec stimulus increased as the n-6:n-3 ratio increased and the interwave I-V interval decreased for a 60 dBnHL 39 clicks/sec stimulus as the n-6 concentrations increased. For both sexes interwave latencies were prolonged for the III-V interwave interval for an 80 dBnHL 39 clicks/sec as n-3 increased.

As the n-3 LCPUFA concentrations increased, the amplitude of the 6000 Hz DPOAE in the right ear increased for both sexes. As the n-6:n-3 ratio increased, the amplitude of the 1500 Hz DPOAE in the left ear decreased for females. No associations were present between n-3, n-6 and the n-6:n-3 ratio and the amplitude of the CEOAE.

DISCUSSION

This study examined the association of plasma LCPUFA concentrations and audiometric measures present at 19 years in the Main Cohort of the SCDS. In this high fish consuming population we found no overall association between LCPUFA and hearing acuity. The mean LCPUFA concentrations were similar if a participant had normal hearing or a sensorineural hearing loss. LCPUFA concentrations were similar regardless of the severity of the hearing loss (mild or moderate) or sex. There were 6 significant associations present between plasma LCPUFA concentrations among the 174 ABR and OAE auditory endpoints studied. Some associations with prolonged ABR latencies or smaller OAE amplitudes would be considered detrimental while others with shortened ABR latencies or larger OAE amplitudes would be considered beneficial. Inclusion of covariates did not influence these results. The associations were present in only one sex, in different measures and appeared to be random. No consistent pattern was seen in the associations identified. These data do not support an association between LCPUFA status and any specific auditory measure.

These analyses support our previous data examining the relationship of these same auditory measures with prenatal and recent MeHg exposures (Orlando et al., 2014). The inclusion of LCPUFA did not change any of the previous findings. These results are also similar to our previous report which showed no association between MeHg exposure and hearing acuity or the severity of hearing loss (Orlando et al., 2014). In the current investigation there were no significant correlation between LCPUFA and MeHg exposure. Both n-3 LCPUFA and MeHg are considered good biomarkers of fish consumption. The lack of a significant correlation between n-3 and MeHg exposure might be related to the different concentrations of each in the various fish consumed and they may measure concentrations over different time periods. Neither biomarker resulted in an association that suggested a clear beneficial or detrimental pattern. For a detailed comparison as a function of prenatal and recent MeHg exposure levels see Orlando et al., (2014).

There is a paucity of data when looking for an association between LCPUFA status and auditory function measures in adults. We do know that early brain development in humans appears to be highly dependent on the availability of LCPUFA (Amin et al., 2000; Yehuda, 2003; Khedr et al., 2004; Unay et al., 2004; Koletzko et al., 2008). Experimental animal studies of low and high concentrations of LCPUFA have reported associations with several detrimental auditory outcomes (Church et al., 2007; Church et al., 2009) . However, translating animal data to humans is challenging because of the physiologic differences, different developmental timeframes, varying dosages of supplementation, ability to control diets and genetics among other factors (Dziorny et al., 2013).

People consume a variety of dietary items most of which contain both beneficial nutrients as well as small amounts or detrimental substances. High dose exposure to MeHg has significant negative health outcomes including hearing loss and other neurologic conditions. To date, however, there is little agreement that lower dose exposure is associated with detrimental effects.

This study has a number of strengths. The cohort is large, participants consumes large amounts of oceanic fish and participants have been longitudinally followed since birth. They do not consume sea mammals and are not exposed to other neurotoxicants. Audiometric testing is objective, was

automated, carried out by specially trained professional staff and scored by an experienced audiologist. All clinical personnel were blinded to LCPUFA status as well as other covariates such as hearing loss and MeHg exposure. The analyses were carried out using an *a priori* analysis plan based on a biological hypothesis, while controlling for confounding factors such as sex and middle ear function that could result in a misinterpretation.

The study also has some limitations. Current plasma LCPUFA status was studied and previous status may have been influential. Plasma LCPUFA status may vary widely depending on dietary intake and this study looked at their concentrations at one time point. The study was observational and there may have been unknown covariates that could influence the results.

In conclusion, we studied the association of LCPUFA status on audiometric measures at age 19 years and found only a few (6) significant associations among a large number (174) of auditory outcome measures. Some of the significant associations would be considered adverse resulting in prolonged ABR latencies or smaller OAE amplitudes. Others significant associations would be considered beneficial resulting in shortened ABR latencies or larger OAE amplitudes. There was no consistent pattern to indicate that current plasma LCPUFA status in a population with high fish consumption are associated with hearing loss or ABR latencies and OAE amplitudes.

Financial Interests

The authors have no competing financial interests to declare.

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Table 1. Means and standard deviations of the absolute wave latencies (wave I, III and V) as a function of presentation level, presentation rate, ear and sex. Values are in msec.

			Females				Males			
			Right Ear		Left Ear		Right Ear		Left Ear	
Level	Rate	Wave	Mean	sd	Mean	sd	Mean	sd	Mean	sd
60 dBnHL	19/sec	I	1.73	0.16	1.71	0.18	1.85	0.19	1.77	0.17
		III	3.82	0.20	3.78	0.21	4.00	0.23	3.97	0.25
		V	5.70	0.23	5.66	0.23	5.98	0.25	5.97	0.30
	39/sec	I	1.77	0.19	1.75	0.21	1.90	0.21	1.80	0.20
		III	3.89	0.25	3.86	0.20	4.10	0.26	4.08	0.26
		V	5.86	0.25	5.82	0.24	6.15	0.27	6.13	0.33
	69/sec	I	1.82	0.22	1.80	0.26	1.98	0.22	1.85	0.17
		III	4.01	0.26	3.98	0.27	4.26	0.32	4.24	0.30
		V	6.10	0.25	6.04	0.27	6.37	0.31	6.37	0.34
80 dB nHL	19/sec	I	1.43	0.11	1.43	0.11	1.50	0.14	1.48	0.17
		III	3.56	0.16	3.55	0.15	3.70	0.21	3.71	0.20
		V	5.39	0.24	5.39	0.20	5.60	0.23	5.62	0.24
	39/sec	I	1.46	0.12	1.45	0.12	1.53	0.16	1.51	0.18
		III	3.62	0.18	3.61	0.18	3.77	0.20	3.76	0.19
		V	5.51	0.23	5.51	0.19	5.74	0.23	5.75	0.25
	69/sec	I	1.49	0.13	1.47	0.13	1.58	0.16	1.55	0.18
		III	3.68	0.18	3.68	0.16	3.84	0.21	3.85	0.24
		V	5.69	0.25	5.68	0.21	5.91	0.26	5.94	0.27

Table 2. Means and standard deviations of Distortion Product OtoAcoustic Emissions and Click Evoked OtoAcoustic Emissions as a function of frequency, ear and sex. Values are the signal to noise ratios in dB.

		Females				Males			
		Right Ear		Left Ear		Right Ear		Left Ear	
Type	Freq(kHz)	Mean	sd	Mean	sd	Mean	sd	Mean	sd
DP	1.0	10.24	8.60	9.38	8.67	9.72	7.88	10.42	7.72

	1.5	16.24	9.44	14.84	10.00	16.02	9.38	15.62	9.06
	2.0	17.69	9.34	15.45	9.95	16.83	9.66	16.31	9.38
	3.0	19.65	8.55	17.86	9.52	19.03	8.29	18.42	8.44
	4.0	21.31	9.00	19.76	9.18	18.94	9.23	19.09	9.50
	6.0	18.39	9.58	17.11	9.85	15.33	8.93	15.44	9.53
CEOAEs	**1.0**	9.00	7.60	8.70	8.00	8.58	7.48	8.16	7.92
	1.5	14.33	7.92	12.73	8.40	12.31	7.93	11.35	8.03
	2.0	15.59	6.99	14.77	7.97	14.35	7.26	13.10	7.80
	3.0	15.69	6.92	14.72	7.24	13.55	6.42	13.38	6.66
	4.0	13.75	6.72	13.17	6.94	10.42	6.19	10.83	6.14

Table 3. Means, standard deviations (sd), and p values of LCPUFA concentrations (n-6, n-3 n-6:n-3 ratio) in the cohort studied as they relate to sex, hearing status, and degree of hearing loss.

Current LCPUFA	**Mean n-6 (sd)**	**Mean n-3 (sd)**	**n-6:n-3 ratio (sd)**
=438	0.15 (0.04)	0.04 (0.01)	3.76 (1.93)
Sex			
Females (N=254)	0.15 (0.04)	0.04 (0.01)	3.76 (1.97)
Males (N=184)	0.15 (0.05)	0.05 (0.02)	3.77 (1.87)
p value	0. 539	0. 779	0.928
Hearing Status			
Normal (N=396)	0.15 (0.04)	0.05 (0.01)	3.70 (1.79)
Hearing Loss (N=42)	0.16 (0.07)	0.04 (0.02)	4.35 (2.88)
p value	0.493	0.416	0.158
Degree of Hearing Loss			
Mild Hearing Loss (N=33)	0.15 (0.03)	0.04 (0.02)	4.26 (2.75)
Moderate Hearing Loss (N=9)	0.20 (0.13)	0.05 (0.01)	4.69 (3.47)
p value	0.252	0.5	0.738

Table 4. Auditory brainstem response regression models. Model 1 uses n-3 and n-6 as the measures of LCPUFA exposure. Model 2 uses the ratio n-6:n-3 as a single measure of LCPUFA exposure. Models with significant interactions between sex and plasma LCPUFA concentrations show separate coefficients for males and females. Coefficients significant at the 0.05 level are displayed in bold.

	Model 1		**Model 2**

Level, Rate	Wave	F/M	n	Model p-value	Sex Male	n-3	n-3 p-value	n-6	n-6 p-value	Post Hg		Model p-value	Sex Male	n-6:n-3 Ratio	n-6:n-3 p-vlaue	Post Hg
80 dB, 19/sec			7			-		-		-						-
			4	0.0	**0.06**	0.3		0.0		**0.0**		0.0	**0.06**	0.0		**0.0**
	I		8	00	**9**	23		40		**03**		000	**8**	01		**03**
			7							-						-
			5	0.0	**0.17**	0.0		0.1		**0.0**		0.0	**0.17**	0.0		**0.0**
	III		5	00	**0**	12		47		**03**		000	**0**	02		**03**
			7							-						-
			6	0.0	**0.23**	0.2		0.2		0.0		0.0	**0.23**	0.0		0.0
	V		1	00	**5**	23		21		03		000	**6**	02		03
			7													
			4	0.0	**0.09**	0.3		0.1		0.0		0.0	**0.09**	0.0		0.0
	I-III		6	00	**5**	22		37		00		000	**6**	01		00
			7											-		
			4	0.0	0.06	0.5		0.0				0.0	0.06	0.0		
	I-V		8	00	5	03		07				000	4	02		
										-						-
										0.0						0.0
		F								05						05
										0.0						0.0
		M								04						04
			7					-						-		
	III-V		5 5	0.0 00	0.01 5	0.1 63		0.1 07				0.0 000	0.01 5	0.0 02		
										-						-
										0.0						0.0
		F								03						03
										0.0						0.0
		M								02						02
80 dB, 39/sec			7							-						-
			3	0.0	**0.08**	0.0		0.0		**0.0**		0.0	**0.08**	0.0		**0.0**
	I		9	00	**0**	02		64		**03**		00	**0**	01		**03**
			7			-				**-**						**-**
			5	0.0	**0.16**	0.5		0.1		**0.0**		0.0	**0.16**	0.0		**0.0**
	III		0	00	**8**	29		11		**03**		00	**7**	04		**03**
			7							-						-
			5	0.0	**0.24**	0.5		0.2		0.0		0.0	**0.24**	0.0		0.0
	V		9	00	**0**	23		06		02		00	**2**	00		02
			7			-		-								
			3	0.0	**0.08**	0.4		0.0		0.0		0.0	**0.08**	0.0		0.0
	I-III		6	00	**1**	89		02		00		00	**0**	03		00

		7					-					-	
		3	0.0	**0.14**	0.5		0.1	0.0		0.0	**0.14**	0.0	0.0
	I-V	8	00	**5**	36		09	01		00	**5**	03	01
		7					-					-	
	III-	4	0.0	**0.06**	**1.0**	**0.0**	0.1	0.0		0.0	**0.06**	0.0	0.0
	V	9	00	**6**	**29**	**318**	12	01		00	**7**	06	01
80 dB, 69/sec		7			-		-	**-**					**-**
		0	0.0	**0.09**	0.1		0.0	**0.0**		0.0	**0.09**	0.0	**0.0**
	I	5	00	**3**	92		29	**04**		00	**2**	00	**04**
		7			-		-	**-**					**-**
		4	0.0	**0.17**	0.1		0.0	**0.0**		0.0	**0.17**	0.0	**0.0**
	III	7	00	**7**	13		46	**03**		00	**7**	02	**03**
		7						-					-
		5	0.0	**0.25**	0.3		0.3	0.0		0.0	**0.25**	0.0	0.0
	V	6	00	**0**	13		87	03		00	**2**	03	03
		7					-					-	
		0	0.0	**0.07**	0.3		0.1	0.0		0.0	**0.07**	0.0	0.0
	I-III	0	00	**5**	88		12	01		00	**5**	02	01
		7											
		0	0.0	**0.13**	0.6		0.0	0.0		0.0	**0.14**		
	I-V	2	00	**6**	04		92	01		00	**7**		
													-
												0.0	0.0
		F										07	03
												-	
												0.0	0.0
		M										15	04
		7											
	III-	4	0.0	**0.06**	0.3		0.0	0.0		0.0	**0.12**		0.0
	V	4	00	**5**	72		72	00		00	**8**		00
												0.0	
		F										04	
												-	
												0.0	
		M										13	
60 dB, 19/sec		5			-			-					-
		3	0.0	**0.25**	0.8			0.0		0.0	**0.08**	0.0	0.0
	I	7	00	**2**	10			02		00	**3**	08	02
							0.6	**0.0**					
		F					**41**	**229**					
							-						
							0.4						
		M					90						
		6						-					-
		8	0.0	**0.19**	0.4		0.4	0.0		0.0	**0.19**	0.0	0.0
	III	6	00	**7**	24		70	03		00	**8**	10	03

		7					-					-
		3	0.0	**0.30**	0.4	0.3	0.0		0.0	**0.30**	0.0	0.0
	V	4	00	**6**	37	06	03		00	**7**	04	03
		5				-	-				-	-
		3	0.0	0.00	1.2	0.0	0.0		0.0	0.00	0.0	0.0
	I-III	3	00	1	51	14	01		00	3	01	01
		5				-					-	
		3	0.0	**0.17**	1.1	0.1	0.0		0.0	**0.17**	0.0	0.0
	I-V	7	00	**7**	27	50	00		00	**9**	03	00
		6			-	-					-	
	III-	8	0.0	**0.09**	0.1	0.2	0.0		0.0	**0.08**	0.0	0.0
	V	4	00	**0**	30	65	01		00	**9**	06	01
60 dB, 39/sec		4					-					-
		2	0.0	**0.19**	0.1	0.1	0.0		0.0	**0.19**	0.0	0.0
	I	7	00	**3**	81	96	03		00	**4**	04	03
		6					-					**-**
		3	0.0	**0.22**	1.0	0.1	0.0		0.0	**0.22**	0.0	**0.0**
	III	1	00	**1**	58	91	04		00	**3**	03	**04**
		7					-					-
		2	0.0	**0.31**	0.5	0.2	0.0		0.0	**0.31**	0.0	0.0
	V	8	00	**6**	46	02	04		00	**7**	04	04
		4				-	-				-	-
		2	0.0	**0.11**	0.6	0.2	0.0		0.0	**0.11**	0.0	0.0
	I-III	3	00	**8**	31	90	01		00	**8**	01	01
		4			-							
		2	0.0	**0.29**	0.1				0.0	**0.18**	0.0	0.0
	I-V	7	00	**0**	84				00	**7**	00	00
						-	-					
						0.0	0.0					
	F					29	06					
						-						
						1.3	**0.0**	0.0				
	M					**24**	**114**	04				
		6			-	-						
	III-	3	0.0	**0.08**	0.7	0.1	0.0		0.0	**0.07**	0.0	0.0
	V	1	00	**0**	15	53	01		00	**8**	02	01
60 dB, 69/sec		2			-		-					-
		9	0.0	**0.12**	0.7	0.0	0.0		0.0	**0.12**	0.0	0.0
	I	6	00	**4**	47	64	04		00	**1**	00	04
		5					-					-
		5	0.0	**0.26**	0.8	0.1	0.0		0.0	**0.26**	0.0	0.0
	III	5	00	**5**	24	56	04		00	**7**	04	04
		7					-					-
		1	0.0	**0.31**	0.2	0.2	0.0		0.0	**0.31**	0.0	0.0
	V	2	00	**4**	17	50	03		00	**4**	05	03

			2				-				-		
			8	0.0	**0.11**	0.4	0.3	0.0	0.0	0.01			0.0
	I-III		7	25	**9**	90	58	02	05	8			02
											-		
											0.0		
		F									13		
											0.0	**0.0**	
		M									**24**	**396**	
			2			-	-						
			9	0.0	0.01	0.4	0.1		0.0	0.00	0.0		
	I-V		6	00	2	12	52		00	9	10		
								-					-
								0.0					0.0
		F						02					02
								0.0					**0.0**
		M						**11**					**11**
			5			-	-						
	III-		5	0.0	0.03	0.8	0.1	**0.0**	0.0	0.03	0.0		**0.0**
	V		3	16	4	12	95	**04**	20	1	00		**05**

Table 5. Otoacoustic emission regression model results. Model 1 uses n-3 and n-6 as the measures of LCPUFA exposure. Model 2 uses the ratio n-6:n-3 as a single measure of LCPUFA exposure. Models with significant interactions between sex and plasma LCPUFA concentrations show separate coefficients for males and females. Coefficients significant at the 0.05 level are displayed in bold.

					Model 1							**Model 2**				
	Freq (kHz)	**Ear**	**F/M**	**n**	**Model p-value**	**Sex Male**	**n-3**	**n-3 p-value**	**n-6**	**n-6 p-value**	**Post Hg**	**Model p-value**	**Sex Male**	**n-6:n-3 Ratio**	**n-6:n-3 p-value**	**Post Hg**
DPOAE				3					-		-			-		-
				5	0.9	0.16	6.4		1.2		0.0	0.8	0.16	0.0		0.0
	1.0	R		8	06	2	21		17		93	30	2	75		93
				3					-					-		
				4	0.7	0.35	25.		1.1		0.1	0.6	0.37	0.1		0.1
	1.0	L		1	01	1	373		16		08	27	8	44		06
				3					-					-		
				5	0.8	0.29	39.		9.2		0.0	0.6	0.29	0.3		0.0
	1.5	R		8	01	8	022		50		03	58	6	63		03
				3					-				-			
				4	0.7	0.10	25.		4.5		0.1	0.3	3.70			0.1
	1.5	L		1	74	1	108		39		18	14	4			08

														-	**0.0**
														0.6	**43**
			F											**71**	**5**
														0.3	
			M											40	
				3		-		-	-			-	-		-
				5	0.6	0.05	17.	0.9	0.0		0.5	0.03	0.0		0.0
	2.0	R		8	80	2	191	50	74		81	8	92		75
				3		-	-					-			
				4	0.9	0.05	20.	1.4	0.0		0.9	0.07	0.0		0.0
	2.0	L		1	78	2	911	65	64		66	2	92		66
				3			-	10.	-						-
				5	0.6	0.76	18.	93	0.1		0.5	0.79	0.2		0.1
	3.0	R		8	20	3	140	4	05		58	1	09		06
				3				-					-		
				4	0.8	0.09	6.2	1.9	0.0		0.6	0.09	0.2		0.0
	3.0	L		1	42	5	49	26	61		60	3	16		62
				3		-	-	15.	-			-			-
				5	0.4	1.77	14.	01	0.0		0.3	1.72	0.2		0.0
	4.0	R		8	58	5	547	4	66		78	4	96		69
				3		-		-				-	-		
				4	0.3	1.19	58.	5.0	0.0		0.3	1.14	0.3		0.0
	4.0	L		1	76	2	481	36	88		93	0	34		81
				3		**-**		**0.0**	-			**-**	-		-
				5	0.0	**2.37**	**86.**	**11**	0.1		0.1	**2.28**	0.2		0.1
	6.0	R		8	31	**9**	**706**	**1**	66		32	**3**	44		09
				3		-	105		-			-	-		-
				4	0.0	2.01	.34	3.4	0.0		0.0	1.87	0.3		0.0
	6.0	L		1	11	6	9	80	25		94	1	70		40
				3		-		-				-	-		
				5	0.9	0.45	12.	4.6	0.0		0.9	0.46	0.2		0.0
	1.0	R		8	92	1	790	50	30		06	2	37		33
				3		-	-					-			
				4	0.6	1.32	2.6	2.7	0.0		0.5	1.31	0.0		0.0
	1.0	L		1	84	3	85	37	58		69	5	62		58
				3		**-**	-	-				**-**	-		
CFOAF				5	0.2	**1.90**	3.3	5.4	0.0		0.1	**1.93**	0.1		0.0
	1.5	R		8	96	**6**	56	34	18		94	**7**	54		21
				3			-	-							
				4	0.0	**4.58**	25.	0.7			0.0	**4.67**	0.1		
	1.5	L		1	12	**4**	894	80			07	**8**	62		
									0.3						**0.3**
			F						**23**						**33**
									-						**-**
									0.2						**0.3**
			M						**98**						**01**

				3		-	-	-	-			-	-	
				5	0.7	1.24	7.5	7.3	0.0		0.7	1.28	0.1	0.0
	2.0	R		8	59	6	34	81	03		22	8	02	01
				3			-							
				4	0.0	**4.82**	27.	6.1			0.0	**4.91**	0.2	
	2.0	L		1	00	**5**	564	06			00	**8**	19	
									0.3					**0.3**
			F						**52**					**59**
									-					**-**
									0.3					**0.3**
			M						**19**					**22**
				3		**-**	-		-			**-**		
				5	0.1	**2.08**	28.	4.8	0.0		0.1	**2.09**	0.1	0.0
	3.0	R		8	58	**5**	872	04	04		60	**8**	07	00
				3		**-**	-					**-**	-	
				4	0.1	**1.78**	6.6	0.6	0.0		0.1	**1.78**	0.0	0.0
	3.0	L		1	72	**0**	44	79	54		13	**6**	42	56
				3			-							
				5	0.0	0.12	18.	5.3			0.0	0.18	0.2	
	4.0	R		8	04	7	231	17			02	1	16	
									0.0					0.0
			F						74					76
									-					**-**
									0.1					**0.1**
			M						**93**					**95**
				3		**-**		10.				**-**		
				4	0.0	**2.52**	7.8	83	0.0		0.0	**2.46**	0.0	0.0
	4.0	L		1	40	**2**	93	8	07		47	**9**	73	04

Figure 1. Absolute Wave I, III and V latencies as a function of presentation level and rate for male and female participants. Standard deviations are extremely small and are not displayed in this figure but can be seen in Table 1