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Serum docosahexaenoic acid and cognitive impairment of Japanese residents in a remote island

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Abstract

Backgrounds: Docosahexaenoic acid (DHA), which is mainly derived from marine fish, is suspected to reduce the risk of dementia and cognitive decline. The associations of fish consumption and DHA with the scores of Kana Pick-out test (KPT), a cognitive function test to detect pre-dementia, were examined in a remote island of Kagoshima, Japan.

Subjects and Methods: As a part of an annual health checkup of residents, a questionnaire survey, hair sample collection, and the KPT were conducted in a remote island of Kagoshima during September and October, 2007. Out of 1,188 residents taking a health checkup, 505 (224 men and 281 women, age 30-69), who received all examinations, were analyzed. Among of them, serum samples from 424 subjects were available for a free fatty acid assay. Hair mercury level was analyzed by cold vapour atomic absorption method, and serum free fatty acid level was measured by gas chromatography. Multivariate linear regression analysis was used to calculate the age-, sex-, and education-level-adjusted KPT scores among different levels of mercury and the ratios DHA to arachidonic acid (AA).

Results: The average of KPT score in women (38.1) was higher than that of men (35.0, $p<0.001$). The scores decreased with age ($p<0.001$) and lower education levels ($p<0.001$) in both men and women. After adjusting for the effects of age, sex, and education level, KPT score was positively related to the DHA/AA (p for trend = 0.034) in the subjects under the age of 60 but this tendency was not observed in those aged 60 or older.

Conclusion: The present study suggested a potential benefit of DHA on cognitive function in the subjects under the age of 60 but further studies for the elderly people are required.

Key Words: Cognitive Impairment; Dementia; Docosahexaenoic acid; Fish consumption; Kana Pick-out Test

Introduction

Fish is a major dietary source of n-3 polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA) which is suspected to reduce the risk of cognitive impairment and dementia.[1] It has been hypothesized that DHA is associated with cognitive change on the basis of its abundance in brain tissue, and evidence from animal experiments demonstrating superior learning and memory performance among DHA-fed rodents.[2] The antioxidant effect by DHA is a possible mechanism of the improvement of cognitive function since the accumulation of lipid peroxide, induced by free radicals, damages cellular function in the aging process.[3]

Higher intake of eicosapentaenoic acid (EPA) and DHA from diet reduced cognitive decline among elder men with normal cognitive function during a 5-year follow-up.[4] Another prospective study showed an inverse association between DHA intake and the risk of Alzheimer disease incidence among elder residents.[5] Kalmijn *et al.*[6] also reported that dietary intake of EPA and DHA was inversely related to the risk of impaired overall cognitive function and speed of cognitive processes in middle-aged subjects. On the other hand, n-3 PUFAs intake was not related to a long-term risk of dementia or Alzheimer disease in a Dutch population aged over 55.[7]

A higher plasma phosphatidyl-choline DHA concentration was associated with a decrease in the risk of developing all-cause dementia in the Framingham study.[8] In the Canadian Study of Health and Aging, however, found no evidence of a reduced risk of dementia or Alzheimer disease among the subjects with higher plasma concentrations of total n-3 PUFAs, DHA, or EPA.[9] The variations in the numerous studies may be due to various factors including study designs, sample sizes, the heterogeneity of study populations, differences in PUFA intake estimations, the serum assays of PUFAs and the wide variety of the applied cognitive tests.

The Kana Pick-out Test (KPT) was developed in Japan as a tool for evaluating frontal lobe function and screening for mild or slight dementia.[10, 11] In this test, subjects read a short story in hiragana, Japanese syllabic characters which children first learn, and, while reading, circle characters that comprise five Japanese vowels within two minutes. Since functional activity of sub-genual cingulated gyrus (SGC) was closely related to scores of KPT,[12] the KPT provides a promising tool for screening to detect early stages of Alzheimer disease with low SGC function, and it is widely used in clinical practice to screen early stage of dementia[13] and to detect cognitive dysfunction in patients with Parkinson's disease.[14] Furthermore, the KPT is also considered as a test suitable for evaluating working memory and executive function as well as for prefrontal-area function.[13]

Prevention of the cognitive deficit among elderly people is one of the critical issues in aged society including Japan. However, epidemiological reports from Asian countries, where the fish-consumption is relatively higher than that in European countries, are quite limited. To evaluate a potential protective efficiency of DHA on cognitive decline in Japanese, the present study examined the association between fish consumption and serum DHA, in relation to KPT score among the residents of a southern remote island in Japan, where the migration in this population is relatively small especially among people aged around 40 or above, and their diet including fish does not vary significantly among seasons since the island is located in a sub-tropical region.

Methods

Study subjects and measuring methods

As a part of an annual health checkup of residents in W-town, a remote island in Kagoshima prefecture, a questionnaire survey, hair sample collection, and the KPT, a cognitive function test, were conducted during September and October, 2007. Among 1,188 residents, age 30-69, taking the health checkup, 774 (65%) participated to this study. Furthermore, 523 (68%) subjects (233 men and 290 women) out of 774 underwent the cognitive test. The participation rate for the cognitive test was 68%, 69%, and 66% for <50, 50-59, and 60-69 age-groups, respectively. The KPT scores of 18 out of 523 subjects (3%) were lower than the age-specific cut-off points for suspected cognitive deficit, as described later, and these subjects were excluded from the analyses. Since the purpose of the present study is to examine the preventive effects of fish consumption / DHA intake on cognitive function, we focused on the subjects with normal cognitive function. Thus, the total number of the subjects was 505. This study was approved by the ethics committee of Graduate School of Medical and Dental Sciences, Kagoshima University (the approval number: 31, 86, and 363). A written informed consent was obtained from all subjects.

In the KPT, examinees were asked to pick out five hiragana letters corresponding to the five vowels used in Japanese language i.e. "A", "I", "U", "E" and "O", while reading a short story written in hiragana in 2 minutes. A score of the KPT was calculated by subtracting the number of wrong letters (other than those 5 vowels) from the number of letters picked out correctly. The maximum correct picking score is 61 in this examination. The age-specific cut-off points for suspected cognitive deficit are as follows: 30, 29, 21, 15, 10, 9, and 8 points for age 20's, 30's, 40's, 50's, 60's, 70's, and 80's, respectively.[13] For the analysis of the association with KPT score, the study subjects were stratified by age 60, <60 and ≥60, since the age-specific cut-off points indicated that the age-dependent decrease of KPT score was the most significant in the population age 30's-50's, and the magnitude of the decrease is small after that.

Information on fish consumption (the amount and frequency of fish consumption), education levels and lifestyles, including smoking and alcohol drinking habits, was collected using a questionnaire. Regarding the frequency of fish intake, interviewees answered one of the following choices; none, once, 2-3 times, 4-5 times, 6-8 times, or ≥ 9 times per week. The volume of fish per meal was determined according to the number of fillet, 4-5 x 4-5 x 1 cm in size, which corresponds to approximately 25g. The amount of fish consumption per week was estimated by multiplying its volume (25, 50, 75, 100, or 125g) and the frequency of intake (0, 1, 2.5, 4.5, 7 or 9 times).

Serum samples were subjected to a free fatty acid analysis. In brief, 200 μL of serum was transferred into a glass tube with a Teflon-lined screw-cap and 200 μL of internal control (tricosanoic acid; 23:0), 200 mg of potassium hydroxide and 2 mL of methanol were added followed by vigorous mixing. After incubation for 1 h at 80oC, 2 mL of hexane was added, and the mixture was vigorously stirred and subsequently at rest for 5 min at room temperature. The upper layer, unsaponified fraction, was discarded and the saponification by hexane was repeated twice. After that, 0.5 mL of hydrochloric acid and 1 mL of methanol were added to the remaining methanol fraction. The mixture was incubated for 1 h at 80oC and 2 mL of hexane was added followed by vigorous mixing. The upper fraction containing fatty acids methyl ester was collected and 1 μL of the sample was applied for the analysis using the Focus GC Gas Chromatography (Thermo Fisher Scientific Inc., U.S.A) on a capillary column SUPELCOWAX™ 10 (SUPELCO, U.S.A), equipped with an auto-injector, auto-sampler, and flame ionization detector, under the following conditions; the oven temperature was programmed 120 oC for 1 min, and from 120oC to 280oC at a rate of 10oC /min, with holding of the final temperature for 23 min. Total run time for one sample was 40 min. The helium carrier flow rate was 1.8 mL/min. The coefficient of variation for the inter- and intra-assays of DHA and EPA was ~8% and ~14%, respectively. The measurement was repeated four times for both assays.

Hair mercury level was examined as a surrogate marker of fish consumption since human exposure to mercury is primarily from the consumption of fish and shellfish. To wet-digest hair samples (10 mg, about 3 cm length), 1 mL mixed solution of nitric acid and perchloric acid (1:1 ratio) and 2.5 mL sulfuric acid were added. The specimens were heated at 200oC for 1 h and cooled under running water. The volume was adjusted 10 mL using distilled water. Total mercury level (methyl mercury in hair) was then measured using cold vapour atomic absorption (CVAA) method. For each run, one reagent blank and two referent solutions were analyzed using the same procedure. The coefficient of variation for mercury measurement was ~5%. The measurement was repeated five times.

Statistical analysis

Multivariate linear regression analysis was used to calculate the age-, sex-, and education-level-adjusted KPT scores and their corresponding 95% confidence intervals among different levels of mercury and the ratios DHA or EPA to arachidonic acid (AA). Spearman's correlation coefficients were estimated between fish consumption and hair mercury level. All data analyses were performed by STATA version 9.2 (Stata Corp. LP).

Results

Table 1 shows the characteristics of the study subjects; 224 men and 281 women. There were significant differences in the distribution of all factors except age between men and women (Table 1). The average of KPT score in women was higher than that of men (Table 2). The KPT scores decreased with age (p<0.001) and lower education levels (p<0.001) in both men and women. There was an increasing trend of KPT scores with alcohol drinking in women (p=0.013) but that was not true in men. Smoking was not associated with KPT scores in analysis, adjusting for age and education levels (data not shown).

The subjects aged 60 or older showed a weak negative association between fish consumption and KPT scores but there was no association in the subjects under the age of 60. On the contrary, KPT scores tended to increase with hair mercury levels in the subjects under the age of 60 although this association was not statistically significant. Such a

Table 1 Characteristics of the study population

	Number (%)			
	Total (n=505)	Men (n=224)	Women (n=281)	P value
Mean age (min, max)	55.1 (30, 69)	55.4 (31, 69)	54.8 (30, 69)	0.489
Education level				
Junior-high school	119 (24)	49 (22)	70 (25)	<0.001
High school	214 (42)	101 (45)	113 (40)	
Junior college/ Training schools *	71 (14)	16 (7)	55 (20)	
University	47 (9)	39 (17)	8 (3)	
Unknown	54 (11)	19 (8)	35 (12)	
Brinkman Index †				
0	366 (72)	102 (46)	264 (94)	<0.001
≤ 300	38 (8)	27 (12)	11 (4)	
> 300 - 600	45 (9)	40 (18)	5 (2)	
> 600 - 1000	28 (6)	28 (13)	0 (0)	
> 1000	24 (5)	24 (11)	0 (0)	
Unknown	4 (1)	3 (1)	1 (0.4)	
Frequency of Alcohol drinking(day/week)				
None	188 (37)	25 (11)	163 (58)	<0.001
1	83 (16)	20 (9)	63 (22)	
2-4	27 (5)	13 (6)	14 (5)	
5-6	63 (12)	46 (21)	17 (6)	
Everyday	102 (20)	92 (41)	10 (4)	
Unknown	42 (8)	28 (13)	14 (5)	

* Professional training schools are included.

† Number of cigarettes per day × years of smoking

‡ P values were obtained by Mann-Whitney U test for age and chi-square test for education level, Brinkman Index, and alcohol drinking.

tendency was not observed in the subjects aged 60 or older (Table 3). The information on the frequencies of processed fish, fish eggs, and fish sausages was also obtained but there was no significant positive association between these fish products and KPT scores (data not shown).

Hair mercury concentration was used as a surrogate marker of fish consumption. The median of hair mercury levels for men and women were 4.23 ppm (range: 0.18-32.7) and 2.4 ppm (0.37-10.3), respectively. Spearman's correlation coefficients for their associations among men and women were 0.176 (p=0.009) and 0.236 (p<0.001), respectively.

Left over serum samples from 178 men and 246 women were available for a free fatty acid assay (Table 4). Multivariate regression analysis revealed that KPT score was positively related to the DHA/AA ratio in the subjects under the age of 60 (p for trend = 0.034). This association remained even after adjusting the effects of smoking and alcohol drinking habits (p for trend = 0.037). Among the subjects aged 60 or older, however, the score was inversely related to the DHA/AA ratio, and the difference between two groups was marginally

Table 2 Distribution of Kana Pick-out Test scores by age, sex, and level of education

	n	Kana Pick-out Test scores Mean* ± SE*	Range (min, max)	*p* value †
Age (year)				*p* for trend
< 50	124	38.4 ± 1.5	25, 58	<0.001
50 - 59	196	36.9 ± 0.4	16, 59	
60 - 69	185	35.4 ± 1.2	11, 58	
Sex				<0.001
Male	224	35.0 ± 0.6	13, 56	
Female	281	38.1 ± 0.5	11, 59	
Education level				*p* for trend
Junior-high school	119	35.6 ± 0.5	13, 50	<0.001
High school	214	37.1 ± 0.4	14, 59	
Junior college/ Training schools	71	38.6 ± 0.5	20, 58	
University	47	40.1 ± 0.8	20, 58	
Unknown	54	32.6 ± 0.9	11, 52	

* Age-adjusted mean and standard error

† *P* for trend derived from multiple regression model adjusted for age, sex, and education (excluding the unknown category)

significant (p=0.111). The KPT score was not associated with EPA/AA ratio in both age groups.

Discussion

Key findings and discussion

In the present study, KPT scores were positively associated with the serum DHA/AA ratio among the subjects under age 60 (p=0.034) but not in the subjects aged 60 or above. Similar trend was also observed in the association with hair mercury levels though there was no statistical significance. These findings can be interpreted as evidence for the beneficial effects of DHA on cognitive function among Japanese under age 60. There was no positive association between KPT scores and serum EPA/AA ratio.

Recently, a study in Japan reported that elderly Japanese with a moderately-high level of serum DHA, but not EPA, showed a lower risk of cognitive decline than those with low serum DHA after 10-year follow-up.[15] Similar findings were also reported in other cross-sectional studies.[16, 17]

A protective effect of DHA was also suggested for severer status of cognitive decline such as Alzheimer disease.18) Other studies reported the fish-consumption effect on dementia or Alzheimer disease.[5, 19] An international cross-sectional study in developing countries found that higher fish intake was inversely associated with the risk of dementia, using 10/66 protocol developed for dementia diagnosis.[20] On the other hand, a longitudinal study conducted in the Netherlands did not observe the inverse association between fish consumption and the risk of dementia or Alzheimer disease.[7]

Although many observational studies indicate that higher level of serum DHA and fish consumption are protective against the risk of cognitive decline, some intervention studies did not confirm this protective effects in cognitively healthy subjects[21, 22] and patients with Alzheimer disease.[23] Since intervention periods of these studies ranged from 12 weeks to 40 months, the evaluation of a long-term effect might be necessary.

DHA is mainly derived from marine fish but it can also be

Table 3 Distribution of Kana Pick-out Test scores by fish consumption and total hair mercury

	All		Age 30-59		Age 60-69	
	N (%)	Adjusted mean (95% CI) †	N (%)	Adjusted mean (95% CI) †	N (%)	Adjusted mean (95% CI) †
Fish consumption (g/week)	492 (100) *		311 (100)		181 (100)	
≤ 50	184 (37)	35.3 (33.8, 36.7)	125 (40)	36.5 (34.8, 38.3)	59 (33)	33.3 (30.9, 35.8)
50 - 150	157 (32)	34.8 (33.7, 36.0)	97 (31)	36.6 (35.3, 38.0)	60 (33)	32.0 (30.1, 33.9)
> 150	151 (31)	34.4 (33.0, 35.9)	89 (29)	36.7 (35.0, 38.5)	62 (34)	30.8 (28.2, 33.3)
p for trend ‡		0.369		0.838		0.128
Hg level (ppm)	505 (100)		320 (100)		185 (100)	
< 1.85	100 (20)	36.1 (34.7, 37.4)	68 (21)	38.3 (36.7, 39.9)	32 (17)	32.3 (29.7, 34.9)
1.85 –	103 (20)	36.4 (35.5, 37.3)	72 (23)	38.7 (37.7, 39.8)	31 (17)	32.5 (30.7, 34.3)
2.65 –	101 (20)	36.7 (36.0, 37.5)	64 (20)	39.1 (38.2, 40.0)	37 (20)	32.7 (31.4, 34.0)
3.65 –	100 (20)	37.1 (36.2, 38.0)	66 (21)	39.5 (38.3, 40.7)	34 (18)	32.9 (31.4, 34.4)
≥ 5.22	101 (20)	37.4 (36.1, 38.8)	50 (16)	39.9 (38.2, 41.7)	51 (28)	33.1 (30.8, 35.3)
p for trend ‡		0.246		0.280		0.712

* Information of fish consumption was missing for 3 men and 10 women.

† The mean scores and their corresponding 95% confidence intervals were adjusted for the effect of age, sex, and education using a multivariate regression model.

‡ *P* for trend derived from multiple regression analysis adjusted for the effect of age, sex, and education.

Table 4 Distribution of Kana Pick-out Test scores by DHA and EPA

	All		Age 30-59		Age ≥ 60	
	N	Adjusted mean (95% CI) †	N	Adjusted mean (95% CI) †	N	Adjusted mean (95% CI) †
DHA/AA* ratio	424 (100)		263 (100)		161 (100)	
< 0.278	141 (33)	36.1 (34.8, 37.4)	89 (34)	37.9 (36.4, 39.4)	52 (32)	33.4 (31.1, 35.6)
0.278 –	141 (33)	36.8 (36.0, 37.6)	85 (32)	39.2 (38.2, 40.1)	56 (35)	32.9 (31.5, 34.3)
≥ 0.397	142 (33)	37.5 (36.2, 38.7)	89 (34)	40.4 (38.9, 42.0)	53 (33)	32.5 (30.2, 34.7)
P for trend ‡		0.176		0.034		0.603
EPA/AA[a] ratio	424 (100)		263 (100)		161 (100)	
< 0.345	141 (33)	37.3 (36.1, 38.6)	91 (35)	39.7 (38.2, 41.1)	50 (31)	33.5 (31.2, 35.8)
0.345 –	142 (33)	36.8 (36.0, 37.6)	85 (32)	39.2 (38.2, 40.1)	57 (35)	32.9 (31.5, 34.3)
≥ 0.487	141 (33)	36.2 (35.0, 37.5)	87 (33)	38.6 (37.1, 40.2)	54 (34)	32.3 (30.1, 34.6)
P for trend ‡		0.277		0.394		0.498

* DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; AA: Arachidonic acid

† The mean scores and their corresponding 95% confidence intervals were adjusted for the effect of age, sex, and education using a multivariate regression model.

‡ P for trend derived from multiple regression analysis adjusted for the effect of age, sex, and education.

synthesized by humans from dietary alpha-linolenic acid (C18.3). However, the conversion efficiency of alpha-linolenic acid to DHA is quite low; on the order of 1% in infants, and considerably lower in adults.[24)]

Although fish contains various beneficial nutrients, it is also a potential source of dietary intake of methylmercury.[25)] A few epidemiological studies reported adverse effects of methylmercury, through fish consumption, on cognitive function among adults.[26, 27)] Carta *et al.*[26)] compared cognitive function between middle-aged habitual consumers of fresh tuna (median of methylmercury level in whole blood: 41.5 μg/L; controls 2.6 μg/L), and found that methylmercury level was inversely related to the color, word reaction time and digit symbol reaction time. Another study from Taiwan reported that blood methylmercury level was significantly associated with the risk of impairments in remote memory, mental manipulation, and orientation among residents living near a deserted chloralkali factory.[27)] The mean blood methylmercury concentration in this population was 15.3 μg/L. On the other hand, Weil *et al.*[28)] found no relationship between blood mercury level and cognitive function among populations with very low blood level of total mercury, around 2-3 μg/L. These observations are consistent with a suggestion by Masley *et al.*[29)]: a higher level of blood mercury, particularly ≥15 μg/L, overwhelms the beneficial effects of n-3 FA. Measuring hair mercury level is a choice for biological monitoring of human environmental methylmercury exposure,[30)] and hair methylmercury level is highly concentrated, approximately 250 times of blood level.[31)] The medians of hair mercury level in our study population were 2.895 and 3.41 ppm for the subjects under the age of 60 and aged 60 or older, respectively, and these values correspond to 11.6 and 13.6 μg/L in blood. Although the levels of mercury in the present study are much lower than the recognized concentrations for neurotoxicity, the lack of association between DHA/AA and KPT scores in the subjects aged 60 or above could be partially explained by a relatively higher mercury level in this group.

Regarding the KPT, the age-specific cut-off points and the distribution pattern indicated that this test is sensitive to detect the decline of cognitive function in the population age 30's-50's but that may not be effective for the population aged 60 or older because the magnitude of the age-dependent decrease is small, and the most elderly people are deviated to low scores without a peak.[11,13)] Further studies are necessary to confirm the benefit of DHA among elderly people.

In our study population, there was no positive association between KPT scores and EPA/AA ratio. This finding was consistent with the result of recent study in Japan.[15)] Another study, conducted in the southern island of Japan, reported that serum EPA levels were not related to fish consumption in both men and women.[32)] The Canadian Study of Health and Aging, a large longitudinal study, reported that serum EPA level was not related to the risk of dementia or Alzheimer disease.[9)] Other prospective studies from Chicago[5)] and Rotterdam[7)] also found no association between EPA intake and the risk of Alzheimer disease and dementia, respectively.

The absence of the association between serum EPA level and cognitive function is consistent with the fact that brain EPA levels are significantly lower than DHA and AA.[33,34)] The low EPA level in the brain could be explained by multiple mechanisms such as beta-oxidization,[35,36)] elongation/desaturation of EPA into longer n-3 PUFA, and lower recycling rate within brain phospholipids.[37)] Chen *et al.* reported a rapid and extensive beta-oxidization of EPA in the brain [35,36)] and a significantly low rate of EPA recycling in brain phospholipids in comparison with DHA and AA.[37)] These findings indicate that EPA may not play an important role in the brain.

The strengths and limitations of the study

The ratio of DHA or EPA to AA, applied in the analysis, is a better indicator than concentrations of DHA and EPA because dietary intake of n-3 PUFA is known to reduce the amount of AA in phospholipids by diminishing its synthesis and simple physical replacement.[38)] Furthermore, Hossain *et al.*[39)] suggested that the cerebral DHA/AA ratio was a good indicator of the host defense capability against oxidative damage.

In general, erythrocytes, in which n-3 PUFA persists for months, are preferable to determine the exposure to DHA/EPA since the regulation of free fatty acids is less stable than that in either in serum lipoprotein particles or in erythrocytes.

A cross-sectional study design was one of the important limitations in the present study. Although we tried to ask long-term lifestyle patterns in the questionnaire, interviewees might answered the recent lifestyle patterns, which could violate the temporality of the causal association. However, for most of the study subjects, aged over 40, their dietary preferences are likely to stay constant.

In the present study, the frequency and the volume of fish consumption in the highest group might be underestimated because of fixed choices in the questionnaire. We assumed that the highest frequency and volume of fish intake were 9 times per week and 125 g per meal, respectively, even though some subjects might have eaten fish more frequently. This might weaken the association between fish consumption and KPT scores. Furthermore, EPA and DHA content vary widely among species but the information on type of fish was not collected. Although we assumed that the diet in this study area does not change significantly among seasons, the questionnaire survey for the diet was conducted only once. We cannot deny a possibility of seasonal variation of fish consumption.

Conclusion and the implications for future research

A beneficial effect of DHA on cognitive function was confirmed in the present study but it was limited to middle-aged population, under age 60. Although further studies using erythrocytes, in which n-3 PUFA persists for months, are warranted, a prospective study would provide further clarification on the association between DHA and cognitive function in the elderly.

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本邦離島住民における血清ドコサヘキサエン酸と認知機能との関連について

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背景：魚介類から主に摂取されるドコサヘキサエン酸（DHA）には、認知症のリスクおよび認知機能低下を予防する可能性があることが報告されている。本研究では、鹿児島の離島地域住民を対象として、早期認知症のスクリーニングとしても用いられるかなひろいテストの得点と魚介類の摂取量および血清 DHA 値との関連を検討した。

対象と方法：鹿児島県の離島において、2007 年 9 月、10 月に住民健康診断を受診した者を対象とし、魚介類の摂取などに関する質問調査、毛髪の採取およびかなひろいテストを健診会場で行った。1,188 名の受診者の中で、すべての検査が実施できた 505 名（男性 224 名、女性 281 名、年齢 30-69 歳）を今回の解析対象とした。さらにその中で血清中の不飽和脂肪酸が測定できた者は 424 名であった。毛髪水銀の測定は冷蒸気原子吸光法で行い、血清中不飽和脂肪酸の測定はガスクロマトグラフィーを用いて行った。年齢、性および教育歴を調整し、かなひろいテスト得点と、魚食量、毛髪水銀値および血清 DHA（アラキドン酸 [AA] との比）などとの関連について多変量回帰分析を行った。

結果 : かなひろい得点の平均値は女性 38.1、男性 35.0 で、女性の方が高かった（P 値：<0.001）。男女とも、かなひろい得点は年齢とともに減少し（P 値：<0.001）、教育歴（年数）と正の関連を示した（P 値：<0.001）。これらの要因を調整した結果、60 歳未満では、DHA:AA 比とかなひろいテスト得点に有意な正の関連を認めた（傾向性の P 値：0.034）が、60 歳以上ではそのような傾向は見られなかった。

結論 : 本研究結果により、60 歳未満の者においては、DHA による認知機能改善効果の可能性が示唆されたが、高齢者における有用性はさらなる検討が必要と思われる。