BioFactors 13 (2000) 41–45
IOS Press


Original report

# Anti-stress effects of DHA

Tomohito Hamazaki[a,*], Miho Itomura[a], Shigeki Sawazaki[b] and Yoko Nagao[c]
[a]*Department of Clinical Application, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama-shi, Toyama 930-0194, Japan*
[b]*First Department of Internal Medicine, School of Medicine, Toyama Medical and Pharmaceutical University, Toyama-shi, Toyama 930-0194, Japan*
[c]*Kogakkan University, Ise-shi, Mie, Japan*

**Abstract.** DHA is abundant in the brain. Deficiency of DHA changes behavior in animals. The purpose of the present studies was to clarify the effect of DHA intake on hostility and plasma catecholamines. In study 1, forty-one students took either DHA-rich oil capsules containing 1.5–1.8 g DHA/d (17 females and 5 males) or control oil capsules containing 97% soybean oil plus 3% fish oil (12 females and 7 males) for 3 mon in a double blind fashion. They took a psychological test (P-F Study) at the start and end of the study. Study 1 started at the end of summer vacation and ended in the middle of mental stress of final exams. In the control group, hostility measured by P-F Study was significantly increased at the end of the study as compared with that measured at the start ($+58\%$), whereas it was not significantly changed in the DHA group ($-14\%$). In a similar double blind two-mon study (study 2), we measured plasma catecholamines and cortisol of students (3 females and 4 males for the DHA group and the same numbers for the control) at the start and end of the study. In study 2 the students were under a continuous stress of final exams that lasted for two mon throughout the whole study period. The plasma cortisol did not change in either group, but the norepinephrine concentration was significantly decreased in the DHA group ($-31\%$), whereas it stayed at the same level in the control group. These effects of DHA intake may be applied to people under psychological stress.

## 1. Introduction

The behavior of both animals and humans is affected by dietary fatty acids. Mice fed an $n-3$ fatty acid-deficient diet and an $n-3$ sufficient diet for two generations had different general behaviors as well as sensitivities to behavior-affecting drugs [1]. Moreover, there is an association between the blood $n-3$ fatty acid status and behavior problems in children. Investigating 6–12 y-old boys, Stevens et al. [2] showed that a greater number of behavior problems, temper tantrums and sleep problems were reported in subjects with lower total $n-3$ fatty acid concentrations in the plasma phospholipid fraction.

In this context we performed series of randomized placebo-controlled double-blind studies using young Japanese students in order to investigate the effects of $n-3$ fatty acids on behavior (Study 1) and plasma catecholamines (CA) (Study 2).

*Correspondence to: Department of Clinical Application, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani Toyama-shi, Toyama 930-0194, Japan. Tel.: +81 76 434 7615; Fax: +81 76 434 5057; E-mail: hamazaki@ms.toyama-mpu.ac.jp.

## 2. Study 1 [3]

### *2.1. Subjects and methods*

*Subjects.* Fifty-three students (19–30 years of age) were recruited from two classes of the two universities, Toyama Medical and Pharmaceutical University (TMPU) and Yokkaichi University. The subjects were allocated to either control or docosahexaenoic acid (DHA, $n-3$) group in a double-blind manner. They were asked to take 10–12 capsules (according to subjects' body weights) containing either DHA-rich fish oil or control oil for 3 mon for the DHA and control groups, respectively. Each capsule contained 300 mg of oil and 0.3% $\alpha$-tocopherol. The DHA-rich fish oil contained 49.3% DHA. Consequently, in the DHA group, subjects ingested 1.5–1.8 g DHA/d from capsules. The control oil was a mixture of 97% soybean oil and 3% of another fish oil to camouflage fish odor of DHA capsules. Subjects were asked to complete food frequency questionnaires. Fasting blood was taken for fatty acid analysis. Mental and psychological tests were performed at the start and end of the study.

*PF Study, and Stroop and Dementia-detecting tests.* PF Study was originally developed by Rosenzweig [4]. This psychological test consisted of 24 pictures illustrating frustration. Subjects were asked to look at those pictures and describe their first reactions. The frequency of hostile reactions were measured and regarded as their hostility. Our Stroop test was a test to measure accuracy and speed of instantaneous judgment of the meaning or color of a color-meaning word written in a different color.

Dementia-detecting test was originally created by Kaneko et al. [5]. This test required two kinds of ability at the same time, namely to pick out certain indicated letters from several sentences composing a short story and understand the story itself.

*Statistical analysis.* Data are expressed as means $\pm$ SD. Paired t-test was used for intragroup comparisons of the results at start and end of the study; intergroup differences were analyzed by two-way ANOVA. Differences of $p < 0.05$ were considered significant.

### *2.2. Results*

Improper subjects such as those taking less than 70% of capsules were excluded before the double-blind code was broken. The final numbers of subjects to analyze were 19 (12 females and 7 males) and 22 (17 females and 5 males) for the control group and DHA group, respectively. DHA concentrations in the DHA subjects increased in the total serum phospholipid fraction ($3.1 \pm 0.5\%$ to $6.1 \pm 1.6\%$, $p < 0.001$), whereas those in the control group did not change significantly ($3.6 \pm 1.3\%$ to $3.5 \pm 1.1\%$).

The scores of Stroop and dementia-detecting tests were improved over the study period both in the control and DHA groups. However, there was no significant difference by ANOVA between the two groups for either Stroop or dementia-detecting tests. As shown in Fig. 1, hostility in the control group increased significantly ($+58\%$), but it was not significantly changed in the DHA group ($-14\%$). There were highly significant differences between the two groups.

The averaged daily intake of DHA from food was $220 \pm 90$ mg in the control group and $230 \pm 90$ mg in the DHA group.

### *2.3. Discussion*

We started this study on September 4, around the end of summer vacation, and terminated the study on December 4. For all subjects in TMPU, there were 24 very stressful final exams beginning just a few days after the study ended. In Yokkaichi, all subjects had to finish their graduation thesis (the toughest

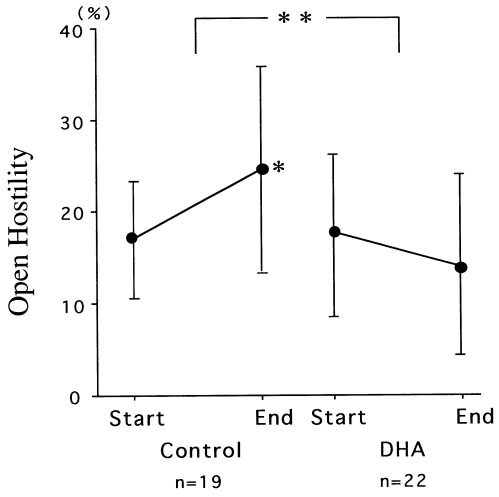


Fig. 1. Changes in Hostility. In the control group, hostility increased because of the stressor (final exams and graduation thesis). *: $p < 0.01$, **: $p < 0.002$.

trial for them) by the middle of December. Consequently, December 4 was one of the busiest and most frustrating days for both TMPU and Yokkaichi subjects. Increased hostility at the end of the study in the control group was highly likely due to those stressful periods in the two universities.

Virkkunen et al. [6] investigated the fatty acid composition of plasma phospholipids in habitually violent and impulsive male offenders. Docosapentaenoic acid ($22:5\,n-6$), the presence of which may indicate $n-3$ fatty acid deficiency, was found in those subjects, but found as trace in control subjects; moreover, DHA levels in offenders were lower than those of controls. These data including ours suggest that $n-3$ fatty acid deficiency might increase behavioral problems, and that DHA administration might solve them.

## 3. Study 2 [7]

### *3.1. Subjects and methods*

We further investigated the effect of DHA on plasma CA levels during a long-term psychological stress. We used the whole period of the final exam period in TMPU lasting 2 mon as a stressor in Study 2. In this study, subjects were asked to take 10 capsules of DHA-rich fish oil or mixed plant oil for 2 mon in a double-blind manner. None of the subjects ($n = 18$) had participated in any fish oil-intervention studies before. At the start (a few days before the first exam of the final exam period) and end of the study (a few days before the last exam), fasting subjects were asked to sit quietly with an indwelling needle in an antecubital vein for 30 min. Then blood was taken for plasma CA and cortisol concentrations and for the fatty acid composition in RBC.

The plasma ratio of epinephrine (EP) to norepinephrine (NE) was log-transformed for normalization. Statistical analysis was performed as in the case of Study 1.

### *3.2. Results*

After excluding 4 improper subjects, we decided to analyze 7 DHA subjects (3 females and 4 males) and 7 controls (3 females and 4 males). DHA in the total phospholipid fraction of RBC increased in

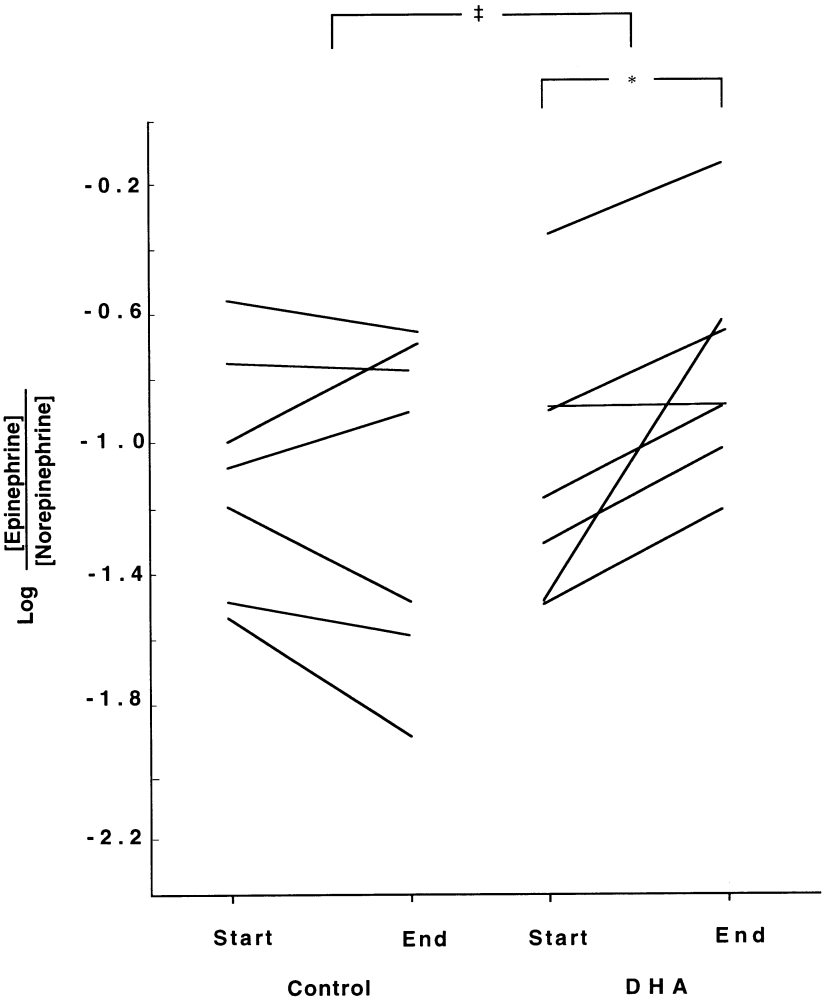


Fig. 2. Changes in the Ratio of Plasma Epinephrine to Norepinephrine. The ratio of plasma epinephrine to norepinephrine was increased in every DHA subject. *: $p < 0.03$, **: $p < 0.02$.

every DHA subjects with the mean values being $5.8 \pm 1.1\%$ to $8.2 \pm 1.1\%$ ($p < 0.002$), whereas in the control group there were no significant changes in any fatty acids between the start and end of the study.

The plasma NE levels significantly decreased in the DHA group ($2.3 \pm 0.8$ nmol/L to $1.6 \pm 0.5$ nmol/L, $-31\%$, $p < 0.03$), whereas it did not change ($2.0 \pm 0.7$ nmol/L to $1.8 \pm 0.7$ nmol/L) in the control group. EP levels decreased in the control group ($187 \pm 121$ pmol/L to $166 \pm 177$ pmol/L) and increased in the DHA group ($197 \pm 149$ pmol/L to $224 \pm 156$ pmol/L), although these changes were not significant in either group. Consequently, the ratio of plasma EP to NE increased in all DHA subjects by 78% (Fig. 2), and there was a significant difference in changes in the ratio between the two groups. There were no significant differences in plasma cortisol levels between the two groups.

### *3.3. Discussion*

Christensen et al. [8] measured basal plasma CA levels of 412 males of 70 years of age and followed them for 7 years. Analysis of plasma CA levels revealed that only 10% of the subjects with high plasma

EP levels and low NE had died during the 7 year-follow up period, but 50% of the subjects with low plasma EP and high NE had died during the same period. Consequently, it is likely that subjects in the DHA group adapted with chronic psychological stress more favorably than for the control group in terms of CA levels.

## 4. Conclusion

Plasma NE is one of the best indicators of the sympathetic nervous system (SNS) tone, and is enhanced by stress. Considering the effect of DHA found in Studies 1 and 2 together, it might be possible that DHA has an anti-stress property controlling behavior on one hand and SNS tone on the other hand.

## Acknowledgments

This work was partly supported by grants from the Japan-United States Cooperative Medical Science Program, the Special Coordination Funds for Promoting Science and Technology of the Science and Technology Agency of the Japanese Government.

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