

# Suicide Attempt and n-3 Fatty Acid Levels in Red Blood Cells: A Case Control Study in China

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**Background:** *Epidemiologic studies show that low fish intake is a risk factor of suicidality; however, there are no case-control studies investigating suicide attempt risk and tissue n-3 fatty acid levels.*

**Methods:** *We recruited 100 suicide-attempt cases and another 100 control patients injured by accidents who were admitted to three hospitals affiliated with Dalian Medical University in Dalian, China. Case and control subjects were matched for age, gender, and smoking status. Those who were inebriated at the time of hospitalization were excluded. Blood was sampled immediately after admission to a hospital. Washed red blood cells (RBCs) were obtained, and the fatty acid composition of the total RBC phospholipid fraction was analyzed by gas chromatography.*

**Results:** *Eicosapentaenoic acid (EPA) levels in RBC in the case subjects were significantly lower than those of the control subjects ( $.74 \pm .52\%$  vs.  $1.06 \pm .62\%$ ,  $p < .0001$ ). When the highest and lowest quartiles of EPA in RBC were compared, the odds ratios of suicide attempt was .12 in the highest quartile (95% confidence interval: .04–.36,  $p$  for trend = .0001) after adjustment for possible confounding factors*

**Conclusions:** *Our findings suggest that low n-3 fatty acid levels in tissues were a risk factor of suicide attempt. Further studies including intervention with fish oil are warranted.*

**Key Words:** Docosahexaenoic acid, eicosapentaenoic acid, fatty acid composition, fish intake, phospholipids, suicide attempt

Suicide is one of the leading causes of premature death around the world. In China, it is the leading cause of death among young adults aged 15–34 years, and the annual suicide rates were estimated to be 23 per 100,000 between 1995 and 1999 (Phillips et al 2002). The incidence might be higher, possibly as high as 30 per 100,000 according to the World Health Organization (Murray et al 1996). In contrast to Western countries, it has been repeatedly documented that suicide rates in China are high among young people in rural areas, particularly among young rural women (Pearson et al 2002). The most important cause of suicide attempt in China is probably acute stress (Li et al 2001). There are no strong religious or legal prohibitions against suicide in China, so people might consider suicide an acceptable method to be freed from stress (Phillips et al 2002). In addition, mental illnesses such as depression, the most important factor in Western countries, may not be the main cause of suicide in China (Chinese Medical Association 1990; Pearson et al 2002). In this context, investigations of suicidal behavior in China have certain advantages. It may be possible to evaluate newly postulated risk factors there more easily, because the influence of psychiatric disease may be less dominant. Moreover, alcoholism, which is one of the well-known risk factors of suicidal behavior (Mann 2003) and disturbs the metab-

olism of a wide range of substances including fatty acids (Pawlosky et al 2001; Salem et al 1997), is still less popular and more tractable in China than in Western countries, especially in women (Wei et al 1999).

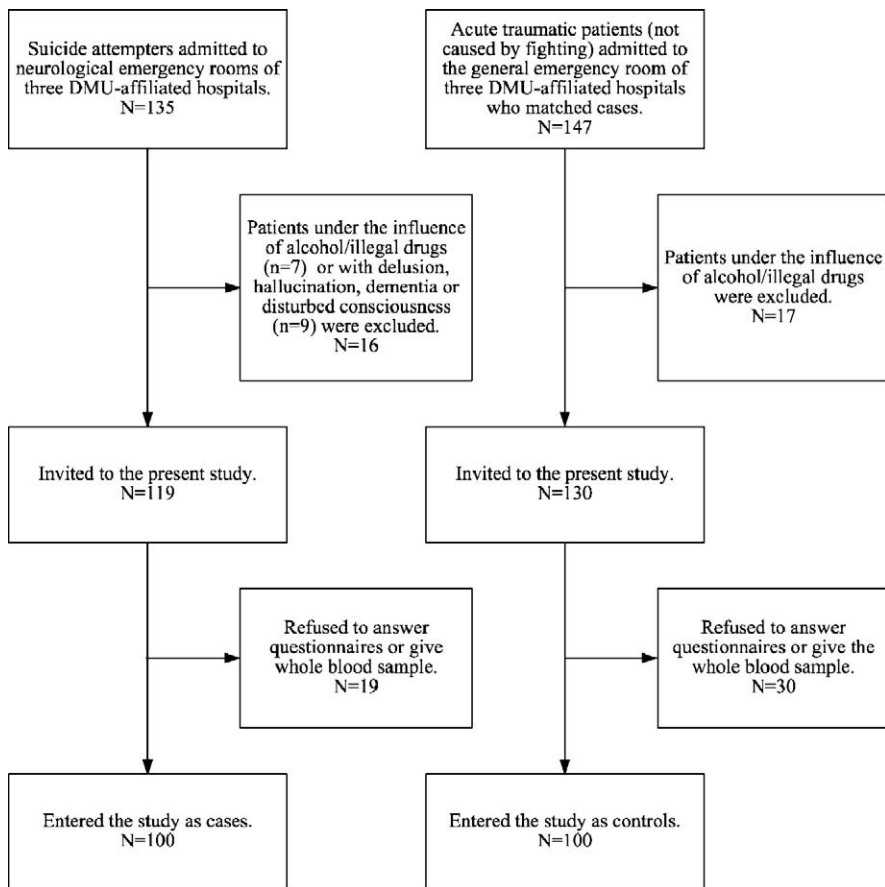
In the United States, more than two thirds of those completing suicide did so on their first attempt (Mann 2002), which means that prevention of secondary suicide attempts alone cannot reduce suicide rates to a considerable degree; however, clinical and neurobiological study of failed suicides can inform us about completed suicide because the two populations are similar, both clinically and demographically (Mann 2003). According to Greer et al (1971), the retrospective research of 204 patients who had attempted suicide for a mean follow-up period of 18 months revealed that about one quarter of them again attempted suicide and that 2% of them actually killed themselves. The increased rate of success by those attempting has persisted over two decades (De Moore and Robertson 1996; Jenkins et al 2002). Understanding the risk factors of suicide attempt is also important in this respect.

Tanskanen et al (2001) conducted a questionnaire study of the relationship between fish consumption and suicidality with 1767 subjects aged 25–64 in a general population of Kuopio, Finland, and found that the risk of suicidal ideation was significantly lower among frequent consumers of lake fish than more infrequent consumers, with an odds ratio (OR) of .57 in a multiple logistic model adjusted for 13 possible confounding factors. In the largest epidemiologic study in Japan, which for 17 years followed more than 260,000 Japanese of both genders aged 40 years and older, Hirayama (1990) reported that the relative risk of suicide was .81 (90% confidence interval [CI]: .72–.91) in subjects who consumed fish daily compared with those who did not. A concise review by Brunner et al (2002) covers the relationship of lipids and suicide and includes consideration of related disorders.

We performed a case-control study with subjects who had attempted suicide and control subjects matched for age, gender, and smoking status to see whether low eicosapentaenoic acid (EPA) levels in red bloods cells (RBCs) were a risk factor of suicide attempt.

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**Figure 1.** Flow chart of subject recruitment. DMU, Dalian Medical University.

## Methods and Materials

### Subjects

The recruitment of case and control subjects was conducted between April and July 2002. During that period, 135 patients who had attempted suicide were sent to neurologic emergency rooms of three hospitals affiliated with Dalian Medical University (DMU): the Second Hospital of DMU, Dalian Friendship Hospital, and Dalian Central Hospital. Those who attempted suicide were not transferred to the neurologic emergency rooms if there was life-threatening danger. One hundred patients who had attempted suicide agreed to participate in the study. Another 100 trauma patients, matched for gender, age in 3-year intervals, and current smoking habits with one of the suicide attempt patients, were individually recruited as control subjects from the general emergency rooms of the same hospitals. Recruitment of the respective control was done within 1 week from the date of the suicide attempt. Drinking status was not considered when matching the case and his or her control because our study protocol made it highly unlikely that there were many patients with drinking problems (as discussed further later in the article). Suicide attempt patients were defined as those who were sent to the department of neurologic emergency because of acute deliberate self-harm, including poisoning. Suicide attempt patients who could not be interviewed because of delusion, hallucination, dementia, or disturbed consciousness were excluded from the study. Those under acute influence of alcohol or illegal drugs and those with traumas of unknown cause were also excluded from the study. (The patients with unknown cause of trauma were all under the influence of alcohol or illegal drug and

thus were excluded regardless of trauma cause). Those patients who were injured in fights were also excluded. Presence of exclusion criteria were determined by attending psychiatrists. Alcohol use was determined by observation of the general behavior and characteristics, including flushed face, smell, and questioning the patient. Illegal drug users were detected by interview. Of the 100 patients who volunteered as control subjects, 91 had been injured at construction sites, factories, or their homes. The rest ( $n = 9$ ) were injured in car accidents. None of the control traumas were judged to be intentional by the physicians in charge. In Dalian, most patients with trauma resulting from car accidents are sent to special hospitals. Therefore, the number of car accident patients was small, and their injuries were not serious. Figure 1 is a subject recruitment flow chart. Local institutional review boards and the ethics committee of Toyama Medical and Pharmaceutical University approved the study, and written informed consent was obtained from each study subject or his or her guardian after full verbal and written explanation.

### Assessment

After a general interview, subjects were asked about the frequency of their fish consumption and asked to choose one of the following categories: at least once a week, more than once per 2 months, and no more than once per 2 months. With regard to patients who had attempted suicide, the 24-item Hamilton Rating Scale for Depression (HRSD; Hamilton 1960) was administered by one of three psychiatrists (MH, HL, YS), followed by the Suicide Intent Scale (SIS; Beck et al 1974). Questions regarding the frequency and method of suicide attempt were

added to the SIS. Suicide attempt was judged impulsive when the scores of items 6 and 15 were null, nonimpulsive when a patient had a maximum score (4), and otherwise judged intermediate. Blood was collected from all study subjects immediately after admission to the hospital, and RBCs from 5 mL of the blood sampled with ethylenediamine tetraacetate were used for fatty acid analysis. The RBCs were washed twice with saline, and the white blood cell layer was removed; RBCs were stored at  $-80^{\circ}\text{C}$  until analysis. The fatty acid composition of the total phospholipid (PL) fraction of RBC was determined by gas chromatography. Briefly, the total lipids were extracted from RBC according to the method of Bligh and Dyer (1959), and the total PL fraction was separated by thin-layer chromatography. Fatty acids of that fraction were transmethylated and analyzed by gas chromatography (a GC-14C gas chromatograph [Shimadzu, Kyoto, Japan], with a capillary column DB-225 [30 m, J&W Scientific, Folsom, California]). The peak areas were recorded with a C-RGA recorder (Shimadzu), and area percentage was calculated. The intraassay coefficients of variance for EPA and docosahexaenoic acid (DHA) were 18% and 6%, respectively.

To obtain objective measures of habitual alcohol consumption of study subjects, aldehyde dehydrogenase (ALDH) in RBC was assayed (Towell et al 1986). This was done after determination of the fatty acid composition of RBC. The remaining RBCs of 20 subjects in each group were thus insufficient for enzyme assay.

The primary outcome measures of our study were the associations between n-3 fatty acid levels in RBCs and the risk of suicide attempt.

### Statistical Analysis

The significant difference between case and control subjects was detected with a chi-square test for categorical variables and unpaired *t* test for continuous variables. The distribution of EPA or DHA in all subjects was used to compute the cutoff points for quartiles of these fatty acids. The association between the level of EPA or DHA and the risk of suicide attempt was expressed as an odds ratio, using the lowest quartile as the reference group. Tests for trend across quartiles of fatty acids were performed by assigning the median value for each quartile to all subjects in that group. Multiple logistic regression analysis was used to control for known and potential risk factors. One-way analysis of variance (ANOVA) was used to detect differences in EPA and DHA levels in RBC among subgroups of various suicide methods. Associations between the EPA levels in RBC of cases and the HRSD or SIS scales were estimated by multiple linear regression analysis. Statistical analyses were performed with StatView software (SAS Institute, Cary, North Carolina).

### Results

Table 1 shows the characteristics of 100 suicide attempt patients and 100 control subjects. There were more wage-earners and students in the control group than in the suicide attempt group. Family income of the control group was larger than that of the suicide attempt group. There was no significant difference in the frequency of fish intake between the suicide attempt and control subjects.

The fatty acid levels in the total PL fraction of RBC are shown in Table 2. The mean levels of EPA, DHA, and total n-3 fatty acids were significantly lower in the suicide attempt group than in the control group. In contrast, the levels of saturated fatty acids, monounsaturated fatty acids, and n-6 polyunsaturated fatty acids did not differ significantly between the two groups except for

**Table 1.** Sociodemographic Characteristics of the Study Subjects

Characteristic	Suicide (n = 100)	Control (n = 100)	p Values <sup>a</sup>
Age (years)			1.0 (matched)
<20	9	7	
20–29	39	39	
30–44	32	29	
45–59	10	15	
60–74	8	9	
≥75	2	1	
Gender			Matched
Male	29	29	
Female	71	71	
Only child	41	30	.10
Had a Physical Disability	6	1	.054
Had a Family History of Attempted suicide	5	1	.10
Marital Status			.13
Never married	44	34	
Currently married	39	55	
Divorced or separated	8	4	
Widowed	9	7	
Formal Schooling			.9
≤6 years	15	17	
7–12 years	44	45	
≥13 years	41	38	
Occupation			<.0001
Wage-earner or student	61	91	
Agricultural laborer	13	5	
Housewife, retired, or unemployed	26	4	
Family income/head/month			<.0001
≤180 RMB	31	7	
181–999 RMB	28	18	
≥1000 RMB	41	69	
Missing data	0	6	
Residence			.2
City/town	81	88	
Village	19	12	
Fish intake			.7
≥Once/week	30	29	
<Once/week but ≥ once/2 months	28	33	
<Once/2 months	42	38	
Current Smoker	22	22	Matched

All data were based on the subject's report. RMB, renminbi (Chinese currency).

<sup>a</sup>p values were calculated by a chi-square test.

stearic acid, which was slightly but significantly higher in the suicide attempt group.

Table 3 shows the odds ratios of suicide attempt among quartiles of EPA levels in RBC PL with assignment of the risk 1.00 to the lowest quartile group (the reference group). The relative risk of suicide attempt in the highest quartile of EPA was about one eighth of the reference group with *p* for trend < .0001. This trend did not change appreciably after adjustment for age and gender (Model 1) or for known and potential risk factors as follows: age, gender, only child, physical disability, family history of attempted suicide, marital status, formal schooling, occupation, monthly per-head household income, residence, and smoking condition (Model 2). A similar relationship was found

**Table 2.** Fatty Acid Levels in the Total Phospholipid Fraction of Red Blood Cells

Fatty Acid	Percentage of Total Fatty Acids		<i>p</i> Values
	Suicide ( <i>n</i> = 100)	Control ( <i>n</i> = 100)	
<b>Saturated</b>			
16:0 <sup>a</sup>	23.4 ± 3.0	23.3 ± 2.9	.95
18:0	14.4 ± 1.8	13.7 ± 1.8	.016
20:0	1.4 ± 1.2	1.4 ± 1.1	.88
22:0	1.8 ± .6	1.7 ± .5	.10
24:0	3.9 ± 1.0	3.9 ± 1.0	.56
Subtotal	44.8 ± 4.3	44.1 ± 4.7	.27
<b>Monounsaturated</b>			
16:1	.19 ± .11	.2 ± .12	.23
18:1 n-9	11.6 ± 1.5	11.5 ± 1.5	.89
20:1	.91 ± .66	.79 ± .67	.20
24:1	3.4 ± .8	3.6 ± .9	.08
Subtotal	16.1 ± 1.4	16.2 ± 1.7	.72
<b>n-3 unsaturated</b>			
18:3 n-3	1.8 ± 1.1	1.8 ± 1.1	.98
20:5 n-3 (EPA)	.74 ± .52	1.06 ± .62	<.0001
22:5 n-3	1.5 ± .5	1.7 ± .5	.004
22:6 n-3 (DHA)	4.4 ± 1.6	5.3 ± 1.7	.0003
Subtotal	8.5 ± 2.4	9.9 ± 2.9	.0002
<b>n-6 unsaturated</b>			
18:2 n-6	13.1 ± 2.3	12.1 ± 1.9	.10
20:3 n-6	1.5 ± .6	1.4 ± .6	.29
20:4 n-6(AA)	11.2 ± 2.1	11.3 ± 2.3	.82
22:4 n-6	2.2 ± .7	2.1 ± .7	.20
22:5 n-6	.49 ± .29	.51 ± .33	.56
Subtotal	28.4 ± 3.7	27.8 ± 3.8	.25
n-6/n-3	3.6 ± 1.1	3.0 ± 0.9	<.0001

Values in columns 2 and 3 are means ± SD. The un-paired *t* test was used.

<sup>a</sup>The number before the colon indicates the number of carbons in the fatty acid; the number after the colon indicates the number of double bonds.

between the DHA levels in RBC PL and the risk of suicide attempt (Table 3).

We did not include any cases under acute alcoholic influence when recruiting subjects; however, we found five drinkers in the suicide attempt group through interview. They were not heavy drinkers; the heaviest drinker consumed 500 mL of beer daily. The mean activity of ALDH of RBC in the suicide attempt group (.127 ± .074 units/g hemoglobin (Hb), *n* = 80) was not significantly different from that in the control group (.122 ± .068 units/g Hb, *n* = 80). The mean values of the suicide attempt patients who stated that they habitually drank alcohol were .063 ± .033 units/g Hb (*n* = 5). We recalculated the odds ratios of suicide attempt subjects without those whose ALDH values were <.063 units/g Hb and reported habitual drinkers. The results were essentially identical to Table 3 (data not shown).

The methods of suicide attempts (*n* = 100) and mean values of EPA and DHA were as follows: taking drugs or poison (.81 ± .51 and 4.5 ± 1.6 for EPA and DHA, respectively, *n* = 52), cutting with a knife (.71 ± .65, 4.2 ± 1.5, *n* = 22), jumping (.64 ± .36, 4.0 ± 2.0, *n* = 8), hanging (.66 ± .59, 4.9 ± 1.8, *n* = 7), gassing (.53 ± .31, 4.3 ± 1.1, *n* = 4), traffic injuries (.93 ± .23, 5.4 ± 1.1, *n* = 4), and other (.38 ± .05, 4.3 ± 1.4, *n* = 3). One-way ANOVA of EPA and DHA among the seven methods of suicide attempts showed no significant results (*p* = .7 and .8, respectively). For 71

suicide attempt patients, this was the first attempt. The rest (*n* = 29) had made a previous attempt(s). There were no significant differences in EPA or DHA levels between first and multiple suicide attempters (data not shown).

In the suicide attempt group (*n* = 100), the number of cases who were very depressed (score > 35) was 24 according to HRSD. The mean score of SIS in the suicide attempt group was 16.7 ± 10.7. Suicide attempt was judged impulsive (*n* = 26), intermediate (*n* = 33), and nonimpulsive (*n* = 41). There were no significant regressions between the HRSD, SIS, or impulsivity scores (combined scores of items 6 and 15 of SIS), and EPA levels after adjustment for either age and gender or the possible confounding factors shown in model 2 in Table 3 (data not shown). Those analyses with DHA levels revealed no significant associations (data not shown).

## Discussion

The most important finding in our study was that there was an eightfold difference in suicide attempt risk between the lowest and highest RBC EPA level quartiles. It is not clear whether low n-3 fatty acid levels in RBC, which may suggest low levels of EPA (Berlin et al 1998) and DHA (Makrides et al 1994) in the central nervous system, caused suicidal behavior; however, low n-3 fatty acid levels in tissues might have cause–effect relationships with suicide attempt through dysfunction of the serotonergic system. First, ample evidence indicates that dysfunction of the serotonergic system is a considerable risk factor of suicidality (Mann 2003). Second, low n-3 fatty acid levels in tissues may depress the serotonergic system; plasma DHA and arachidonic acid concentrations were positively correlated with 5-hydroxyindole acetic acid (5-HIAA, the major metabolite of serotonin) levels in the cerebrospinal fluid (Hibbeln et al 1998). Also, dysfunction of the serotonergic neuron is involved in depression and aggression (Mann 2003), which may be induced by low levels of n-3 fatty acids. Depressed subjects with normal C-reactive protein values had lower n-3 fatty acids in the PL fraction than control subjects in an elderly population in Rotterdam (Tiemeier et al 2003). A few groups of researchers have already reported that pure EPA (Nemets et al 2002; Peet et al 2002) or fish oil (Su et al 2003) significantly ameliorated symptoms of major depression in a double-blind manner. Furthermore, we reported that DHA-rich fish oil consumption controlled student aggression during a period of high stress (students' term and final exams; Hamazaki et al 1996). In a double-blind test, DHA-rich foods also controlled aggression in attention-deficit/hyperactivity disorder (ADHD; Hamazaki and Hirayama 2004). On the other hand, in animal experiments, supplementation with arachidonic acid (AA) and DHA increased the concentration of serotonin in the frontal cortex of piglets (de la Presa Owens and Innis 1999), and depletion of n-3 fatty acids increased 5-HT receptor density in frontal cortex of rats (Delion et al 1996). Interestingly, similar up-regulation was reported among suicide victims (Stanley and Mann 1983). Taken together, it is possible that the serotonergic system is regulated by tissue levels of n-3 fatty acids.

The level of stearic acid (18:0) was significantly higher in the suicide attempt group (Table 2). Peet et al (1998) also reported that stearic acid levels were significantly higher in RBC membranes of depressive patients than those of control subjects. Saturated fatty acids are viscous and probably decrease membrane fluidity (Hamazaki et al 1985), which might affect behavior; however, a relationship between stearic acid levels and suicide attempt has yet to be reported.

**Table 3.** Odds Ratios for Attempted Suicide, According to Quartile of n-3 Fatty Acids in Red Blood Cell Phospholipid Fraction

	Quartile				p for Trend
	1	2	3	4	
<b>EPA</b>					
Range (% of fatty acid)	.10–.47	.47–.73	.74–1.20	1.22–4.16	
Median (% of fatty acid)	.35	.58	.94	1.57	
Case (n)	37	26	24	13	
Crude OR	1.00	.38	.32	.12	<.0001
95% CI		.16–.88	.14–.75	.05–.30	
Model 1	1.00	.37	.32	.12	<.0001
95% CI		.16–.87	.14–.75	.05–.30	
Model 2	1.00	.39	.23	.12	.0001
95% CI		.14–1.09	.08–.66	.04–.36	
<b>DHA</b>					
Range (% of fatty acid)	.56–3.72	3.74–4.80	4.89–6.13	6.15–8.94	
Median (% of fatty acid)	2.71	4.18	5.36	6.86	
Case (n)	32	29	25	14	
Crude OR	1.00	.78	.56	.22	.0003
95% CI		.35–1.74	.25–1.25	.09–.51	
Model 1 <sup>a</sup>	1.00	.76	.55	.22	.0003
95% CI		.33–1.73	.25–1.24	.09–.50	
Model 2 <sup>b</sup>	1.00	.73	.41	.21	.002
95% CI		.26–2.04	.17–1.31	.07–.60	

CI, confidence interval; OR, odds ratio.

<sup>a</sup>Model 1 is adjusted for age and gender.

<sup>b</sup>Model 2 is adjusted for age, gender, only child, physical disability, family history of attempted suicide, marital status, formal schooling, occupation, monthly per head income in family, residence, and smoking condition.

Postmortem investigations of suicide victims have revealed that there were fewer noradrenergic neurons in the locus ceruleus (Arango et al 1996), higher tyrosine hydroxylase (the rate-limiting enzyme in the biosynthesis of norepinephrine) levels in the brainstem, and lower levels of postsynaptic adrenergic receptors in the cortex (Ordway 1997). These findings may suggest an increased stress response before suicide, resulting in excessive release of norepinephrine, a secondary up-regulation in tyrosine hydroxylase biosynthetic activity, and a down-regulation of postsynaptic adrenergic receptors in the cortex (Mann 2002). Depletion of norepinephrine may lead to hopelessness and suicidal behavior (Mann 2003). We have two lines of experimental data suggesting that the central noradrenergic system might be depressed by DHA-rich foods. In our recent intervention study with ADHD children (Hirayama 2004), visual short-term memory and errors of commission in the continuous performance test were not improved in a group consuming DHA-rich foods compared with a control group; those parameters in the control group apparently were significantly improved by learning effects, with significant intergroup difference. These deteriorating effects of DHA on attention suggested its suppressive action on the central noradrenergic system, because psychostimulants, the activator of the noradrenergic system, are frequently prescribed to ADHD patients to reduce their symptoms (Biederman and Spencer 1999). The other line of data is reduction of plasma norepinephrine concentrations after fish-lipid administration (Hamazaki et al, in press; Sawazaki et al 1999). Although peripheral norepinephrine (NE) values do not directly reflect its central activity, both peripheral and central NE activities often vary together (Svensson 1987). The effect of NE on suicide has not been thoroughly investigated, but severe anxiety or agitation is associated with noradrenergic overactivity and higher suicide risk (Fawcett et al 1997). If n-3 fatty acids prevent

excessive NE outputs and NE depletion, the association between n-3 fatty acids and risk of suicide attempt might in part be explained by stabilization of the central noradrenergic system through prevention of NE depletion.

The distribution of impulsivity in the suicide attempt group appeared to be on the less impulsive side compared with that of 39 suicide attempts with neither major depression nor alcohol dependence referred to a general hospital in Helsinki (18 impulsive, 14 intermediate, and 7 nonimpulsive; Suominen et al 1997). The mean SIS score in the suicide attempt group ( $16.7 \pm 10.7$ ) was higher than that of the same nondepressive, nonalcoholic group ( $11.0 \pm 6.7$ ; Suominen et al 1997).

We could not find any significant association between RBC n-3 fatty acids and scores of HRSD, SIS, or impulsivity. These results were contrary to our expectations based on the serotonin and noradrenaline theories described earlier. At present, it is not clear why we could not find any associations. The characteristics of Chinese suicide attempters are different from those of Western countries, with fewer suicide attempts caused by major depression and the major cause of suicide being acute stress (Li et al 2001). If we had measured parameters related to acute stress, we might have been able to find an association between n-3 fatty acids in RBC PL and degrees of acute stress in suicide attempt patients. Interestingly, our previous study with students showed antistress effects of fish oil (Hamazaki et al 1996). We suggest that n-3 fatty acids may control suicidal behavior through reduction of acute stress. Reduction in stress may be related to beneficial changes in the central serotonergic and noradrenergic activities by n-3 fatty acids, although there is only circumstantial evidence for this relation (Hamazaki, in press).

Chronic alcohol consumption is reported to reduce brain DHA concentrations in rhesus monkeys (Pawlosky et al 2001). Long-chain polyunsaturated fatty acids were actually reduced in

the blood of alcoholic subjects (Salem et al 1997). It is well known that alcoholism is a risk factor of suicidal behavior (Berglund and Ojehagen 1998); however, the confounding effects of alcohol appeared to have minimal or no effect on our study, given that there were only five drinkers in the suicide attempt group. Activity of ALDH in RBC is reduced in alcoholics (Musshoff and Daldrup 1998), but it returns to normal ranges after supervised abstinence (Lin et al 1984). The results of recalculating odds ratios excluding subjects with very low ALDH levels also suggested that the effects of alcohol were negligible. Smoking is also a risk factor of suicidal behavior (Breslau et al 1993) and affects the fatty acid composition of RBC. Smoking schizophrenia subjects had lower RBC EPA and DHA levels and lower dietary intakes of  $\alpha$ -linolenic acid, EPA, and DHA than did nonsmoking schizophrenia subjects (Hibbeln et al 2003). In our study, we matched case with control subjects for age, gender, and smoking habits. Moreover, the percentage of smokers was only 22% in both groups. These factors made it likely that smoking did not confound the crude association between n-3 fatty acid levels and suicide attempt risk.

The most frequent method of suicide attempt was drug or poison ingestion. Barbiturates and diazepam were the most frequent drugs used by subjects in our study. The results shown in Table 3 could not be explained by the hypothesis that poisons or drugs reduce EPA and DHA concentrations in RBC because there were no significant differences in EPA or DHA among various types of suicide methods.

There were no associations between the frequency of fish intake and the risk of suicide attempt. Fish consumption is not popular in China, even in cities like Dalian located along the coast (Wang et al 2003). As shown in Table 1, the frequency of fish consumption was low, particular if one compares this figures with Japan's consumption, where more than 90% of the population eats fish two to three times a week or more (Hirayama 1990). In fact, the EPA concentrations in RBC in our study were considerably lower than those of Japanese people of similar age (about 1.4%, not published data), which were similar to the median value of EPA in the highest quartile of our study. In China, no food questionnaire programs are available yet for fatty acid intake. Therefore, we used our own simple questionnaire about the frequency of fish consumption; however, asking about the frequency of fish consumption may not be a good method to estimate a subject's intake of n-3 fatty acids in Dalian or elsewhere in China, because Dalian residents usually eat at a round table and help themselves from a common plate, so the concept of a single portion for one person does not exist. Chinese who eat only a very small bite of fish once a week, might nonetheless indicate on the questionnaire that they eat fish every week. This is one reasons we could not find any association between the frequency of fish consumption and the risk of suicide attempt. We did not ask about fish species in our questionnaire. Two of the popular fish in Dalian, flounder and croaker, are lean and fat, respectively. Fish consumption data without accounting for species may confound the results (Oomen et al 2000).

Defective plasma antioxidant defenses have been reported in major depression. For example, Maes et al (2000) reported that patients with major depression had significantly lower serum vitamin E concentrations than healthy control subjects. If suicidal ideation is also associated with low plasma antioxidant defenses, EPA and DHA levels in RBC may be decreased; however, this hypothesis is unlikely because the level of AA that is susceptible to oxidants was not decreased in the RBC PL fraction in the

suicide attempt group (Table 2). The same reasoning is relevant to the confounding effects of alcohol consumption, because AA is among the most frequently decreased polyunsaturated fatty acids in alcoholic RBCs (Salem et al 1997).

In conclusion, in our study, low levels of EPA and DHA in RBCs were a risk factor of suicide attempt. We suggest that there may be a cause-effect relationship between them. Further studies including intervention were warranted.

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