Essential Fatty Acids Predict Metabolites of Serotonin and Dopamine in Cerebrospinal Fluid among Healthy Control Subjects, and Early- and Late-Onset Alcoholics

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Background: *Impulsive violence, suicide, and depression are strongly associated with low concentrations of cerebrospinal fluid 5-hydroxyindoleacetic acid (CSF 5-HIAA). Increased suicide and trauma reported in some cholesterol-lowering trials may be related to altered concentrations of polyunsaturated fatty acids rather than cholesterol, a possible surrogate marker.*

Methods: *CSF 5-HIAA and homovanillic acid (HVA), total cholesterol, and plasma fatty acid concentrations were examined in 176 subjects, including 49 healthy volunteers, and 88 early- and 39 late-onset alcoholics.*

Results: *Among each group, polyunsaturated fatty acids predicted both CSF 5-HIAA and CSF HVA concentrations, but total cholesterol was unrelated to either neurotransmitter metabolite. The relationships between plasma 22:6n3 and CSF 5-HIAA were significantly different when healthy volunteers ($r = .35$) were compared to early-onset alcoholics ($r = -.38$) ($p < .0002$).*

Conclusions: *Dietary studies are indicated to determine if essential fatty acid supplementation can influence central nervous system serotonin and dopamine metabolism and modify impulsive behaviors related to these neurotransmitters.* Biol Psychiatry 1998;44:235–242 © *1998 Society of Biological Psychiatry*

Key Words: Alcoholism, cholesterol, docosahexaenoic acid, polyunsaturated fatty acids, serotonin, suicide, violence, 5-hydroxyindoleacetic acid, arachidonic acid, essential fatty acids

Introduction

A well-established finding in biological psychiatry is an association between low 5-hydroxyindoleacetic acid (5-HIAA) concentrations in cerebrospinal fluid (CSF) and an increased risk of attempted and completed suicide (Roy et al 1991). Low CSF 5-HIAA concentration, an indicator of reduced serotonin turnover rate in the frontal cortex (Stanley et al 1985), has also been reported to be associated with impaired impulse control (Mann 1995), unprovoked violent and aggressive behavior (Virkkunen et al 1994a), and hostility (Linnoila et al 1983). Muldoon et al (1990) observed that mortality due to suicide, homicide, and trauma appear to increase in therapeutic trials designed to lower plasma cholesterol. These two sets of findings prompted Engelberg (1992) to propose that lowering plasma cholesterol may reduce CSF 5-HIAA concentrations. Indeed, when cholesterol plasma concentrations were lowered by reducing cholesterol intake as an isolated dietary variable, rhesus monkeys became more aggressive while CSF 5-HIAA concentrations were reduced (Kaplan et al 1994).

We postulated that plasma concentrations of polyunsaturated essential fatty acids, rather than plasma cholesterol concentrations, would predict CSF 5-HIAA concentrations. An initial report of low plasma cholesterol concentrations among violent offenders also reported a deficiency in the essential fatty acid docosahexaenoic acid (22:6n3) and replacement with 22:5n6 (Virkkunen et al 1987). Docosahexaenoic acid (22:6n3), is selectively concentrated in neuronal membranes and appears critical for proper neuronal function (Salem et al 1986). Since polyunsaturated essential fatty acids also lower plasma cholesterol measures, they may be an important variable linking drug and dietary therapies that lower plasma cholesterol with increased suicide, homicide, and trauma-related mortality (Hibbeln and Salem 1995). Essential fatty acid plasma concentrations may reach deficient levels when fat intake is reduced by National Cholesterol Education Panel Step 2 diets (Meydani et al 1993; Siguel and Lerman 1994), and diets deficient in 22:6n3 can decrease human brain concentrations of docosahexaenoic acid (Farquharson et al 1992). In rats, an n-3 fatty acid deficient diet resulted in a 44% increase in serotonin$_{2A}$ receptor density in the frontal cortex (DeLion et al 1994). Strikingly similar observations were reported in the frontal cortex of suicide victims, a 44% increase in serotonin$_{2A}$ receptor density (Stanley and Mann 1983).

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In this cross-sectional observational study of healthy volunteers and abstinent alcoholics, we quantified plasma total cholesterol nonessential and essential fatty acids and CSF serotonin and dopamine metabolite concentrations obtained simultaneously under stringently controlled conditions on a locked research ward. Alcoholics were selected for study because of the strong association of suicidal and aggressive behaviors with a greater genetic risk of alcohol dependence described among alcoholics with an early onset of dependence (Mann 1995). Alcoholics who begin drinking at an early age suffer disproportionately more criminal, social, and clinical consequences of their drinking (Irwin et al 1990). Among early-onset violent Finnish alcoholics, low CSF 5-HIAA concentrations have been associated with a history of suicide attempts and with impaired impulse control, which leads to severe social and criminal consequences of their drinking (Virkkunen et al 1994a, 1994b). A marked genetic vulnerability to develop alcoholism associated with early onset, suicide attempts, criminal acts, and male gender has been described in replicated adoption studies (Sigvardsson et al 1996). This genetic vulnerability may be related to a reduced central serotonin turnover rate (Roy et al 1987). Because early-onset alcoholics appear to be a genetically and clinically distinct subgroup, we also postulated that the relationship between concentrations of plasma essential fatty acids and CSF 5-HIAA may distinguish early-onset alcoholics from late-onset alcoholics and healthy volunteers.

Methods and Materials

Subjects

All subjects were admitted to the inpatient research ward of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health Clinical Center in Bethesda, Maryland. Samples of cerebrospinal fluid used to quantify neurotransmitter metabolite concentrations, and fasting plasma samples used to quantify total cholesterol concentrations and total fatty acid profiles, were obtained simultaneously. Dietary fat intake was not modified. Plasma was available for lipid analysis for 127 out of 131 alcoholics, whose differences in CSF monoamine metabolite concentrations comparing early- to late-onset alcoholics have been reported previously (Fils-Aime et al 1996). Plasma and CSF samples were also available for 49 healthy volunteers. Healthy volunteers had negative alcohol breath tests and urine drug testing, were free of major medical disorders on the basis of history, physical exam, and clinical chemistries, and did not meet criteria for current or lifetime psychiatric or substance use disorders. Detoxified alcoholics met Research Diagnostic Criteria (RDC) for alcoholism (Spitzer et al 1978) and were abstinent for between 21 and 63 days at the time of study, confirmed by random breath and urine drug testing. Alcoholics were diagnosed using the Schedule of Affective Disorders and Schizophrenia–Lifetime (SADS) (Endicott and Spitzer 1978), and healthy volunteers were given either the SADS or the Structured Clinical Interview for DSM-III-R (SCID) (Williams et al 1992). Interviews were blind-rated by a research social worker and one psychiatrist under review by a senior research psychiatrist. Subjects with a lifetime history of a major psychotic illness or bipolar affective disorder were excluded. Early-onset alcoholics were defined by onset of excessive alcohol use prior to their 25th birthday. Age of onset was calculated by subtracting years of excessive alcohol consumption from current age. All subjects were medication free at the time of the study. None had received monoamine oxidase or serotonin reuptake inhibitors in the 3 months prior to the study. Michigan Alcoholism Screening Test (MAST) scores (Strogaad et al 1994) and Hollingshead ratings of socioeconomic class were obtained for all subjects. Information on recent and chronic alcohol consumption was obtained from a structured research questionnaire completed by the subject (Eckardt et al 1978). A Hamilton Depression Rating Scale was completed by two nurses within 1 week of the lumbar puncture for 84 early-onset and 37 late-onset alcoholics. All subjects provided written informed consent approved by the NIAAA Institutional Review Board (protocol #92-AA-0185).

Both healthy volunteers and alcoholics were maintained on a low-monoamine diet (Muscettola et al 1977) for a minimum of 3 days prior to the lumbar puncture and blood sampling. This diet did not restrict fish, meat, poultry, or oil consumption. Lumbar punctures were performed after an overnight fast as previously described (Fils-Aime et al 1996). Fasting blood samples were collected in heparin immediately prior to the lumbar puncture. CSF and plasma samples were frozen at −70°C until analysis, except plasma for cholesterol analysis, which was thawed once for less than 30 min and refrozen.

Neurotransmitter Metabolite Assays

Concentrations of the major CSF metabolites of serotonin (5-HIAA) and dopamine [homovanillic acid (HVA)] were quantified with high-performance liquid chromatography using electrochemical detection (Scheinin et al 1983). Samples from alcoholics were completed in one run, whereas samples from healthy volunteers were assayed in batches. Within- and between-run coefficients of variance were less than 10% as previously reported (Fils-Aime et al 1996; Scheinin et al 1983).

Plasma Fatty Acid Composition

Fatty acids were extracted from 200 μL of plasma by a method modified from Folch et al (1957). Samples were aliquoted into 2 mL $CHCl_3$, 1 mL BHT-MeOH, and a known quantity of 23:0 methyl ester as an internal standard. One milliliter of 0.2 mol/L Na_2HPO_4 was added after brief vortexing. The samples were capped under N_2 and vortexed again. After centrifugation, $CHCl_3$ was removed and dried under N_2. Samples were methylated with BF_3-MeOH for 60 min (Morrison and Smith 1959). Samples were kept cold and under N_2 throughout analysis to prevent oxidation. Gas chromatography was performed on a Hewlett-Packard (HP) 5890 series II with a flame ionization detector, an autosampler, and a FFAP capillary column (J&W

Table 1. Demographic Variables

	Healthy volunteers	Late-onset alcoholics	Early-onset alcoholics
n	49	39	88
Male	38	33	84
Female	11	6	4
Caucasian	42	35	74
African-American	7	4	14

Scientific). Peaks were identified using authentic standards (NuCheck Prep, MN). Fatty acids were quantified by comparison to peak areas of the 23:0 internal standard. When subjected to thawing and refreezing, within- and between-run coefficients of variance were less than 0.3% and 5%, respectively.

Total Plasma Cholesterol

Total plasma cholesterol was quantified with a colorimetric cholesterol esterase assay (#352, Sigma, St. Louis, MO) using Center for Disease Control authentic standards (#C7921, Sigma, St. Louis, MO). Standard curves were produced in quadruplicate, and samples were assayed in triplicate on an HP 8452 spectrophotometer. When subjected to thawing and refreezing, within- and between-run coefficients of variance were less than 3% and 5%, respectively.

Statistical Analyses

Statistical analyses were computed using Statistica for Windows 1.0 (Statsoft, Tulsa, OK) and Statview 4.1 (Abacus Concepts, Berkeley, CA). Differences between groups were examined with nonparametric Kruskal–Wallis and Mann–Whitney tests. In separate analyses, Pearson product–moment correlations were computed to assess the relationships between each CSF monoamine metabolite concentration and each fatty acid, total cholesterol, liver enzymes, frequency, quantity and years of excessive alcohol consumption, age, height, weight, body mass index (BMI), Hollingshead social class score, and cigarette use. Forward stepwise multiple regression and testing of equality of correlations were computed. Bonferroni corrections for multiple testing were made throughout where appropriate.

Results

Subject Characteristics

Age of onset of alcoholism and years of excessive alcohol consumption were the only descriptive variables that distinguished the three groups from each other (Tables 1–3). Hollingshead scores of socioeconomic class differed only between healthy volunteers (3.7) and early-onset alcoholics (2.8) ($p < .0001$). Similar differences were found in CSF 5-HIAA ($p < .05$) and HVA ($p < .07$) comparing early- to late-onset alcoholics in a two-group comparison, with 4 fewer alcoholics than reported by Fils-Aime et al (1996). The present study found no significant differences in a three-group comparison that included the group of 49 healthy volunteers.

Plasma Fatty Acids

Compared to healthy volunteers, both groups of alcoholics had higher total plasma fatty acid and increases in many individual fatty acid concentrations (Table 4). The univariate correlations between plasma total fatty acid and liver enzyme concentrations were $r = .26$ ($p < .004$) for guanosine triphosphate (GTP), $r = .20$ ($p < .02$) for aspartate transaminase (AST), and $r = .16$ ($p < .05$) for alanine transaminase (ALT) when all alcoholics were considered as one group. Thus, higher total plasma fatty acid concentrations among alcoholics were probably related to hypertriglyceridemia associated with hepatic steatosis and consistent with elevated plasma ALT, AST, and GTP concentrations.

Correlational Analyses

Variables that had no correlation to CSF neurotransmitter metabolite concentrations prior to multiple testing in-

Table 2. Descriptive Variables

	Healthy volunteers	Late-onset alcoholics	Early-onset alcoholics	*H*	HV/EO	HV/LO	EO/LO
n	49	39	88				
Age (years)	37 (15.7)	45.5 (8.8)	36.5 (8.7)	24.1[a]	ns	ns	$p < .00001$
Height (cm)	170 (20)	173 (12)	175 (12)	6.0	—	—	—
Weight (kg)	75.6 (12)	78.7 (13)	77.1 (13)	2.0	—	—	—
BMI (kg/m)	30.8 (4)	26.6 (5)	25.2 (6)	3.7	—	—	—
Cigarette use (# per year)	88 (619)	3708 (2764)	3521 (2891)	61.6[a]	$p < .0001$	$p < .0001$	ns
Antisocial criteria met	0.3 (0.6)	2.0 (1.2)	2.6 (1.4)	46.6[a]	$p < .0001$	$p < .0001$	ns
Hollinghead score	3.8 (0.99)	3.3 (1.3)	2.8 (1.3)	17.4[a]	$p < .0001$	ns	ns

Mean (SD). *H* indicates Kruskal–Wallis *H* value. Mann–Whitney pairwise comparisons (with $p < .002$ chosen because of multiple testing) between healthy volunteers and early-onset alcoholics are designated as HV/EO, between healthy volunteers and late-onset alcoholics as HV/LO, and between early- and late-onset alcoholics as EO/LO. Antisocial criteria met indicates number of RDC criteria for antisocial personality disorder met by a structured interview.

[a] $p < .007$, chosen because of multiple testing.

Table 3. Alcohol-Related Variables and CSF Metabolites

	Healthy volunteers	Late-onset alcoholics	Early-onset alcoholics	*H*	HV/EO	HV/LO	EO/LO
Excessive alcohol consumption (years)	0	9.1 (6.8)	18.7 (8.7)	—	—	—	$p < .0001$
Frequency (days/last 183)	32.3 (36.4)	118.6 (58.7)	127.6 (52.7)	33.0[a]	$p < .0001$	$p < .0001$	ns
Quantity (g alcohol/day)	46.2 (54.9)	172.4 (151.0)	219.7 (122.3)	37.9[a]	$p < .0001$	$p < .0001$	ns
ALT (U/L)	18.5 (6.2)	46.5 (43.7)	46.7 (50.4)	21.8[a]	$p < .0001$	$p < .0001$	ns
AST (U/L)	20.0 (10.8)	42.3 (31.0)	58.4 (74.5)	25.1[a]	$p < .0001$	$p < .0001$	ns
GTP (U/L)	26.5 (15.1)	129.5 (164.7)	134.4 (204.5)	36.3[a]	$p < .0001$	$p < .0001$	ns
CSF 5-HIAA (pmol/mL)	91.2 (34.4)	103.0 (45.6)	86.3 (31.3)	4.3	—	—	—
CSF HVA (pmol/mL)	174.3 (73.2)	170.2 (69.8)	146.4 (57.0)	5.6	—	—	—

Mean (SD). *H* indicates Kruskal–Wallis *H* value. Mann–Whitney pairwise comparisons (with $p < .002$ chosen because of multiple testing) between healthy volunteers and early-onset alcoholics are designated as HO/EO, between healthy volunteers and late-onset alcoholics as HV/LO, and between early- and late-onset alcoholics as EO/LO.
[a] p .006, chosen because of multiple testing.

cluded total plasma fatty acids, liver enzymes, height, weight, BMI, Hollingshead score, cigarette use, years of excessive alcohol consumption, frequency and quantity of alcohol abuse, and Hamilton Depression Rating Scale scores. Total plasma cholesterol did not correlate significantly with CSF 5-HIAA or HVA concentrations after Bonferroni correction for multiple testing. CSF 5-HIAA or HVA concentrations were intercorrelated among healthy volunteers ($r = .77$, $p < .0001$), and late-onset ($r = .66$, $p < .0001$) and early-onset alcoholics ($r = .78$, $p < .0001$) consistent with previous reports (Agren et al 1986). Consistent with our a priori hypothesis, plasma docosahexaenoic acid was positively correlated with CSF 5-HIAA in healthy volunteers (Figure 1).

Plasma docosahexaenoic acid was the only variable that predicted neurotransmitter metabolite concentrations in all three groups at $p < .05$ [healthy volunteers (CSF 5-HIAA, $r = .35$, $p < .05$, CSF HVA, $r = .31$, $p < .05$), late-onset alcoholics (CSF HVA, $r = .32$, $p < .05$), and early-onset alcoholics (CSF 5-HIAA, $r = -.33$, $p < .001$, CSF HVA, $r = -.31$, $p < .001$)] (Table 5). Thus, the test for equality of correlations was only applied to compare the relationships between plasma docosahexaenoic acid and CSF neurotransmitter metabolites and was corrected for multiple testing by using $p < .01$. The relationship of plasma docosahexaenoic acid concentrations to CSF 5-HIAA concentrations was significantly different comparing healthy volunteers to early-onset alcoholics ($p < .0002$). This difference in relationships approached significance comparing early- to late-onset alcoholics ($p < .05$), but was not significant comparing healthy volunteers to late-onset alcoholics ($p < .21$). Similarly, the relationships of plasma docosahexaenoic acid concentrations to CSF HVA concentrations were significantly different comparing healthy volunteers to early-onset alcoholics ($p < .0009$) and approached significance comparing early- and late-onset alcoholics ($p < .02$), but was not significant comparing healthy volunteers to late-onset alcoholics ($p <$

Table 4. Plasma Cholesterol and Essential Fatty Acids Concentrations

	Healthy volunteers	Late-onset alcoholics	Early-onset alcoholics	*H*	HV/EO	HV/LO	EO/LO
Total cholesterol (mg/dL)	174 (37)	179 (49)	175 (50)	0.6	ns	ns	ns
Total fatty acids (μg/mL)	1418 (526)	2072 (735)	1952 (799)	39.4[a]	$p < .0001$	$p < .0001$	ns
Total saturated (μg/mL)	418 (166)	663 (321)	619 (471)	31.4[a]	$p < .0001$	$p < .0001$	ns
Total polyunsaturated (μg/mL)	642 (167)	903 (246)	885 (250)	48.5[a]	$p < .00001$	$p < .00001$	ns
n6/n3 ratio	13.9 (3.6)	13.9 (4.3)	14.5 (3.4)	1.6	—	—	—
20:4n6/20:5n3 ratio	16.0 (7.2)	17.5 (8.1)	19.3 (6.9)	6.2	—	—	—
18:2n6 (μg/mL)	458 (120)	634 (180)	612 (180)	41.6[a]	$p < .0001$	$p < .0001$	ns
20:4n6 (μg/mL)	103 (34)	146 (50)	156 (49)	49.5[a]	$p < .000001$	$p < .00003$	ns
22:5n6 (μg/mL)	3 (2)	5 (2)	6 (2)	48.6[a]	$p < .0003$	$p < .000001$	ns
18:3n3 (μg/mL)	8 (4)	10 (5)	9 (4)	9.9	—	—	—
20:5n3 (μg/mL)	8 (5)	10 (6)	9 (5)	4.1	—	—	—
22:6n3 (μg/mL)	23 (9)	36 (17)	32 (12)	23.5[a]	$p < .00001$	$p < .0003$	ns

Mean (SD). *H* indicates Kruskal–Wallis *H* value. Mann–Whitney pairwise comparisons (with $p < .001$ chosen because of multiple testing) between healthy volunteers and early-onset alcoholics are designated as HV/EO, between healthy volunteers and late-onset alcoholics as HV/LO, and between early- and late-onset alcoholics as EO/LO.
[a] $p < .003$, chosen because of multiple testing.

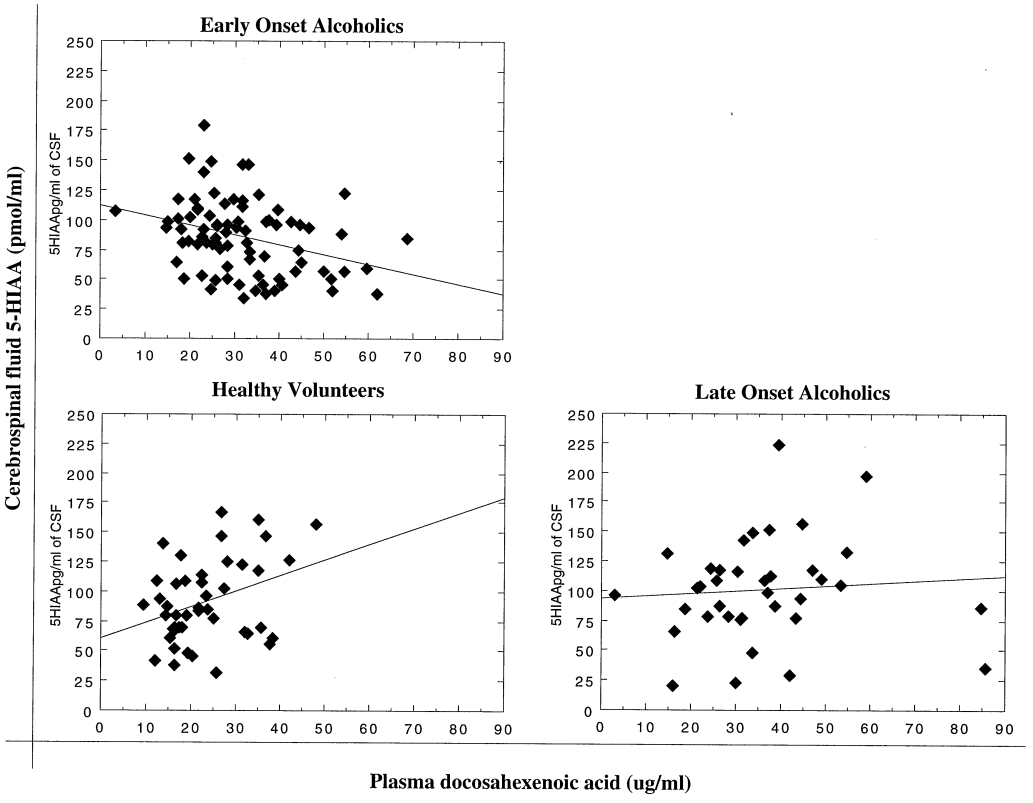

Figure 1. Scattergrams and regression lines for plasma docosahexaenoic acid (22:6n3) and cerebrospinal fluid 5-HIAA in early-onset alcoholics ($r = -.33$, $p < .003$), late-onset alcoholics ($r = .08$, ns), and healthy volunteers ($r = .36$, $p < .05$).

.46). These findings suggest that the relationship between plasma docosahexaenoic acid and CSF 5-HIAA concentrations among early-onset alcoholics is different from this relationship among late-onset alcoholics and healthy volunteers (Figure 1). Two late-onset alcoholics had plasma docosahexaenoic acid and eicosapentaenoic acid concentrations that were more than three standard deviations from the mean. When the data were analyzed excluding these 2 subjects, the relationship between plasma docosahexaenoic acid and CSF 5-HIAA ($r = .36$, $p < .02$) among late-onset alcoholics was similar to healthy volunteers ($r = .35$, $p < .05$). This exclusion would amplify both the differences between the early- and late-onset alcoholics and the similarity between late-onset alcoholics and healthy volunteers; however, these 2 late-onset alcoholics met all inclusion criteria and were retained in the late-onset group.

Table 5. Variables with Correlation Coefficients of $p < .005$ When Correlated with CSF 5-HIAA and HVA

	5-HIAA	*r*	HVA	*r*
Healthy volunteers	20:4n6	.46[a]	20:4n6	.49[b]
			22:5n6	.48[b]
			Age	.42[a]
Late-onset alcoholics	18:3n3	.50[a]	—	
Early-onset alcoholics	22:6n3	−.33[a]	22:6n3	−.31[a]
	20:5n3	−.33[a]		
	22:5n3	−.30[a]		
	LCn6/LCn3	.31[a]		

LCn6/LCn3 is the ratio of the sum n-6 to the sum of n-3, 20 and 22 carbon fatty acids. Groupings indicate sets of independent variables, that is all those with $p < .05$, which were used in forward stepwise multiple regression analysis.

[a] $p < .005$, chosen because of multiple testing.

[b] $p < .001$, chosen because of multiple testing.

Stepwise Multiple Regression

Forward stepwise multiple regression analyses were conducted on models including all variables that predicted CSF 5-HIAA or HVA at $p < .05$. The models were then reanalyzed including liver enzymes, smoking, and frequency and quantity of alcohol consumption (variables that were not significant predictors at $p < .05$); this yielded the same results, which are presented in Table 6. Among healthy volunteers, plasma 20:4n6 positively predicted both CSF 5-HIAA and HVA concentrations. Among late-onset alcoholics, 18:3n3 positively predicted CSF 5-HIAA concentrations; however, among early-onset alcoholics, plasma docosahexaenoic acid was inversely related to both CSF 5-HIAA and HVA concentrations (Table 6).

Table 6. Results of Stepwise Multiple Regression Analysis Using CSF 5-HIAA and HVA Concentrations as Dependent Variables

	5-HIAA	β	model r^2	HVA	β	model r^2
Healthy volunteers	20:4n6	.46 ($p < .002$)	(.21)	20:4n6	.48 ($p < .0008$)	(.29)
				Age	.32 ($p < .03$)	
Late-onset alcoholics	18:3n3	.50 ($p < .002$)	(.26)	—		
Early-onset alcoholics	22:6n3	−.33 ($p < .003$)	(.10)	22:6n3	−.30 ($p < .006$)	(.10)

For each group, the model used for forward multiple stepwise regression included all variables selected as significant at $p < .005$ in predicting CSF metabolites by univariate regression analysis (see Table 5).

Discussion

In this cross-sectional study of healthy volunteers and abstinent alcoholics, CSF concentrations of 5-HIAA were predicted by plasma concentrations of polyunsaturated fatty acids, and in particular by docosahexaenoic acid, an n-3 essential fatty acid selectively concentrated in the brain (Figure 1) (Salem 1989). CSF 5-HIAA is a neurotransmitter metabolite of serotonin that has been repeatedly associated with the pathophysiology of violent, suicidal, and impulsive behaviors (Mann 1995). These correlational relationships remained highly statistically significant after multivariate analyses that included potentially confounding factors such as age, alcohol consumption, and smoking history. It should be clearly noted that this study does not demonstrate that altering dietary intake of polyunsaturated fatty acids will cause alterations in CSF neurotransmitter metabolite concentrations or in behavior. If there is such a causal relationship, then a direct interpretation of these data is that increasing the dietary intake of long-chain polyunsaturated fatty acids may increase CSF 5-HIAA concentrations and reduce impulsive and violent behaviors in subjects similar to healthy volunteers. We caution that this interpretation must be tested in prospective intervention trials; however, several studies are consistent with this interpretation. In a 5-year prospective interventional study, Weidner et al (1992) found that diets rich in n-3 polyunsaturates significantly decreased hostility and depression. Similarly, in several observational studies, low concentrations of n-3 polyunsaturates predicted impulsive behaviors (Stevens et al 1995) and greater severity of depression (Adams et al 1996; Maes et al 1996; Peet et al 1998). Hamazaki et al (1996) demonstrated in a double-blind placebo-controlled trial that docosahexaenoic acid reduced aggression among healthy Japanese students, which was remarkable, as they had high plasma concentrations of n-3 polyunsaturates prior to the intervention.

Our study of plasma fatty acid profiles presumes a corresponding alteration in the nervous system lipid composition. Chronic, moderate alcohol consumption can cause depletion of docosahexaenoic acid from the adult brain of felines and rhesus monkeys (Salem and Ward 1993; Pawlowsky and Salem 1995). This is especially significant because of the association of alcohol use with violent behaviors (Mann 1995). In several neurological disorders, deficiencies in plasma essential fatty acids are a marker for similar losses in brain (Pawlowsky and Salem 1996), and plasma and erythrocyte polyunsaturates also generally correlate with nervous system levels during early mammalian development (Uauy et al 1996).

Possible confounding variables in the relationship between CSF 5-HIAA concentrations and plasma essential fatty acid concentrations included altered food selection, depression, cigarette usage, alcohol consumption, and alcoholic liver damage; however, neither liver enzymes, total plasma fatty acids, measures of alcohol intake, cigarette usage, nor Hamilton Depression Rating Scale scores were correlated with CSF 5-HIAA or HVA concentrations, neither in simple nor in multivariate regression models. Early-onset alcoholics, with lower socioeconomic status, may have had lower dietary intake of fish due to its cost; however, decreased fish intake would not explain why the relationship differed between CSF neurotransmitter metabolites and plasma docosahexaenoic acid concentrations when comparing healthy volunteers to early-onset alcoholics. There is a possibility that residual alcohol-induced hepatic damage might have increased tryptophan oxygenase activity and decreased the availability of plasma tryptophan and thus confounded comparisons of the CSF 5-HIAA concentrations between alcoholic and control groups (Buydens-Branchey et al 1989); however, within 15–21 days of the last drink, alcohol-induced increases in tryptophan oxygenase activity and the lower ratio of tryptophan to other amino acids both returned to stable levels (Buydens-Branchey et al 1989). In this study, all CSF and plasma was collected at least 21 days after the last drink. Nonetheless, increased tryptophan oxygenase activity may potentially account for the lack of differences in CSF 5-HIAA concentrations when comparing the healthy control group to either alcoholic group. Since both groups of alcoholics still had higher total plasma fatty acid concentrations, perhaps the most appropriate group for early-onset alcoholics is late-onset alcoholics, who had no difference in time from the last drink, markers of hepatic damage, MAST scores, or lifelong measures of alcohol consumption. In this comparison, early-onset alcoholics

did have significantly lower CSF 5-HIAA concentrations than did late-onset alcoholics.

In early-onset alcoholics alone, elevated plasma docosahexaenoic acid predicted lower CSF 5-HIAA concentrations. In contrast to the early-onset alcoholics, late-onset alcoholics and healthy controls both had similar positive correlations between plasma docosahexaenoic acid and CSF 5-HIAA, despite having significant differences in markers of hepatic damage and lifetime drinking histories. This finding also distinguished early- and late-onset alcoholics, although the groups had no differences in markers of hepatic damage, lifelong measures of alcohol consumption, plasma docosahexaenoic acid levels, or any other plasma fatty acid variable. Inclusion of potential confounders including age, smoking, and those discussed above, into multivariate regression models did not alter these relationships. Nonetheless, in an attempt to replicate this finding, we next compared violent and nonviolent subjects in groups containing a similar composition of alcoholics and nonalcoholics to further isolate alcohol consumption and hepatic damage as confounding variables in the relationship between plasma essential fatty acids and CSF 5-HIAA and HVA (Hibbeln et al 1998). Violent subjects and early-onset alcoholics had similar negative correlations between plasma docosahexaenoic acid and CSF 5-HIAA (Hibbeln et al 1998).

Thus, our study also suggests that dietary recommendations to increase n-3 polyunsaturated fat intake, if applied to early-onset alcoholics or violent subjects, may result in a decrease in CSF 5-HIAA concentrations, which may be associated with an increased risk of impulsive, violent, and suicidal behaviors (Roy et al 1991; Mann 1995; Virkkunen et al 1994a; Linnoila et al 1983) (Figure 1). The negative correlation observed only among early-onset alcoholics and the violent groups in HVA (Hibbeln et al 1998) may indicate that they exhibit a genetic variant of essential fatty acid transport, metabolism, or selective brain uptake. Alternatively, these subjects may have a genetic variant of essential fatty acid regulation of serotonin synthesis, release, metabolism, or uptake.

In conclusion, this study describes a correlational relationship between polyunsaturated essential fatty acids in plasma and metabolism of serotonin and dopamine in the central nervous system. Since total plasma cholesterol concentrations were not related to CSF 5-HIAA or HVA concentrations, uncontrolled changes in dietary intake of polyunsaturated fatty acids may be responsible for the inconsistent reports of increased suicide and mortality from accidents during cholesterol-lowering therapies. Cholesterol-lowering drugs themselves alter tissue polyunsaturated fatty acid composition (Hibbeln and Salem 1996). We caution that these data have not demonstrated that changes in dietary intake of polyunsaturated essential fatty acids can cause changes in central serotonergic function or alter impulsive or depressive behaviors. We suggest that the role of polyunsaturated essential fatty acids should be considered in studies that examine the hypothesis that plasma lipids are involved in the increased risk of impulsive, depressive, or suicidal behaviors.

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