

Plasma total cholesterol concentrations do not predict cerebrospinal fluid neurotransmitter metabolites: implications for the biophysical role of highly unsaturated fatty acids¹⁻³

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ABSTRACT Low concentrations of a metabolite of serotonin found in cerebrospinal fluid (CSF), 5-hydroxyindolacetic acid (5-HIAA), are strongly associated with suicidal and violent behaviors. Although lowering of plasma total cholesterol has been suggested to increase mortality from suicide and violence by decreasing concentrations of CSF 5-HIAA via changes in membrane biophysical properties, highly unsaturated fatty acids may play a more important role. Violent and nonviolent comparison groups, early- and late-onset alcoholics, and healthy comparison subjects were studied to control for alcohol use and predisposition to violence. Fasting plasma total cholesterol and CSF were assayed under stringently controlled conditions. When all groups were combined ($n = 234$), plasma cholesterol concentrations had a weak positive correlation with CSF 5-HIAA ($r = 0.18$, $P < 0.01$). However, age correlated with both plasma total cholesterol and CSF 5-HIAA concentrations. When age was included in multiple regression models, the correlation between cholesterol and CSF 5-HIAA concentrations was not significant. Cholesterol correlated weakly with CSF 5-HIAA concentrations only in late-onset alcoholics after age was controlled for, but the relation was not significant after correction for multiple testing. CSF homovanillic acid did not correlate with plasma total cholesterol in any group. Plasma total cholesterol had no apparent relation to CSF neurotransmitter metabolites in any group of subjects. Highly unsaturated essential fatty acids, which are also critical determinants of membrane biophysical properties and may be linked to brain serotonin concentrations, should also be considered in studies examining the effect of lowering fat intake on the incidence of suicide and violence. *Am J Clin Nutr* 2000;71(suppl):331S-8S.

INTRODUCTION

The hypothesis that therapies that lower plasma cholesterol also increase mortality from suicide and violent behaviors remains controversial. Some studies on lowering plasma cholesterol have reported increased deaths from suicide, homicide, and accidents (1-3), whereas others have shown no association (4-7). In contrast, 2 reports suggested that reduced hostility (8, 9) and reduced depression (8) were associated with increased consumption of highly unsaturated n-3 fatty acids. One critical hypothesis is that low concentrations of plasma total cholesterol are related to low concentrations of cerebrospinal fluid (CSF) 5-hydroxyindolacetic acid (5-HIAA) via

alterations in physical properties of neuronal membranes (1, 10). Low CSF 5-HIAA has been cited as a mechanism that may link low plasma cholesterol concentrations with increased suicide and violence. CSF 5-HIAA, the principal metabolite of serotonin, strongly predicts serotonin turnover in the human frontal cortex (11). Low concentrations of CSF 5-HIAA have been repeatedly and robustly associated with impulsive behaviors (12), and specifically suicide (13) and violence (14, for review see 15). However, the association between plasma cholesterol and CSF 5-HIAA concentrations was directly tested in only one human study, which unfortunately lacked a control group. In 72 subjects with a history of suicide attempts, no relation was found between plasma total cholesterol and CSF 5-HIAA concentrations, and a weak positive correlation was found between HDL and CSF 5-HIAA concentrations (16), contrary to the prediction that therapies that improve cholesterol profiles might lower CSF 5-HIAA concentrations (1, 10).

Methodologic problems in 2 other studies included the use of indirect measures of central serotonin turnover rate, lack of control for age, alcohol intake, and predisposition to violence, lack of control groups, and small sample size (17, 18). Age (4, 19, 20) and alcohol use (21-24) can be powerful confounders in testing for a relation between plasma total cholesterol and CSF 5-HIAA concentration, depression, or suicide. In a study of elderly subjects, Brown et al (4) found that a weak association between plasma total cholesterol and depression rating scores was not significant after correction for age, self-report of health status, and physical function. In low doses, alcohol can increase HDL concentrations (21), but chronic, excessive consumption sufficient to cause hepatic damage can increase total cholesterol concentrations by disturbing apolipoprotein metabolism (22). Acute intake of alcohol releases serotonin and raises central serotonin concentrations whereas chronic usage depletes central serotonin concentrations (23, 24). Unique relations between plasma cholesterol and CSF 5-HIAA concentrations may exist in subjects predisposed to violence or suicide, such as early-onset alcoholics

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(25) or homicidal offenders, in whom low concentrations of CSF 5-HIAA (26, 27) and low plasma total cholesterol concentrations (14, 28), respectively, have been reported. Moderate alcohol intake can deplete highly unsaturated fatty acids from cortical tissues in adult felines and rhesus monkeys (29, 30). In an attempt to control for these potential confounders, we tested for significant correlations between plasma total cholesterol and CSF 5-HIAA concentrations in subjects with a history of violence, in a comparison group without a history of violence matched for alcohol use, in healthy subjects, and in 2 subgroups of recently abstinent alcoholics. Using these groups, we were able to control for use of alcohol, history of violence, and age in well-characterized subjects in the stringently controlled environment of a clinical research unit.

SUBJECTS AND METHODS

Subjects

All of the above-described subject groups were mutually exclusive. 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, MD, and provided written, informed consent before study under protocols 94-AA-0164 or 83-M-10 as approved by the NIAAA Institutional Review Board. Healthy comparison subjects and violent and nonviolent comparison subjects completed the Structured Clinical Interview for Diagnosis (31). Early- and late-onset alcoholics completed the Schedule for Affective Disorders (32). Interviews were blind-rated by a research social worker and a psychiatrist with subsequent review by a senior research psychiatrist. At the time of the study, all subjects were free of medication for ≥ 3 wk, had negative urine drug screen results, were free of major medical disorders on the basis of history, physical exam, and clinical chemistry results, and did not meet criteria for current or lifetime major psychotic illnesses or bipolar affective disorder. All were free of a lifetime history of regular intravenous drug abuse or current codependence on any drugs. None had received monoamine oxidase or serotonin reuptake inhibitors in the 3 mo before the study. Michigan Alcoholism Screening Test (MAST) scores (33), Hollingshead ratings of socioeconomic class, and Buss-Durkee hostility scales (34) were obtained for all subjects. Information on recent and chronic alcohol consumption was obtained from a structured research questionnaire (35). Detoxified alcoholics met Research Diagnostic Criteria (36) and *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed, revised (37) criteria for alcoholism, had negative urine drug screen results, and had been abstinent from alcohol for between 21 and 63 d at the time of study, confirmed by random breath and urine testing. Healthy comparison subjects had no lifetime history of alcohol dependence in addition to the previously described criteria. Early-onset alcoholics were defined by onset of excessive alcohol use before their 25th birthday. Age of onset was calculated by subtracting years of excessive alcohol consumption from current age. Plasma was available for lipid analysis for 127 of the 131 alcoholics, for whom differences in CSF monoamine metabolite concentrations among early- compared with late-onset alcoholics were reported previously (27). Plasma and CSF metabolite concentrations were also available for 49 healthy comparison subjects, 27 violent subjects, and 31 nonviolent comparison subjects. Violent and nonviolent com-

parison groups were distinguished by their histories of violent behaviors but contained equal proportions of alcohol-dependent subjects, as described below.

Both violent subjects and nonviolent comparison subjects were recruited by newspaper advertisement. Inclusion criteria for violent subjects included having a lifetime history of multiple (>5) episodes of violent physical aggression that had actually caused or had clear potential to cause bodily harm. Examples of these episodes included pushing someone down stairs, punching someone in the face or body, kicking, stabbing, and threatening with a gun in a fit of anger. All subjects reported having recent episodes of violent behavior (within the past 3 mo) or having withdrawn from a significant personal relationship to avoid such episodes. All violent subjects reported at least one episode of violence unrelated to alcohol intoxication or withdrawal. Exclusionary criteria for violent and nonviolent control subjects included a history of seizure or other neurologic disorders, history of head trauma that resulted in loss of consciousness, or a lifetime history of abuse or dependence on cocaine, amphetamines, or other illicit drugs. Thirteen of 27 violent subjects and 13 of 31 nonviolent subjects had a current history of alcohol dependence. Thus, the number of alcohol-dependent subjects in the nonviolent comparison group was similar to that in the violent group. Nonviolent comparison subjects reported having had no episodes of violent physical aggression in their lifetime.

All subjects were maintained on a low-monoamine diet (38) for a minimum of 3 d before the lumbar puncture and blood sampling. This diet did not restrict fish, meat, poultry, or oil consumption. Lumbar punctures were performed as described previously (27). All samples for analysis were prepared from the first 12 mL CSF collected and were frozen at -80°C until analyzed. Both samples of CSF used to quantify neurotransmitter metabolite concentrations and fasting plasma samples used to quantify total cholesterol concentrations were obtained within 2 h of each other from healthy volunteers and early- and late-onset alcoholics and within 1 wk of the lumbar puncture from the violent and nonviolent comparison subjects.

Plasma total cholesterol assays

For healthy comparison subjects and early- and late-onset alcoholics, plasma total cholesterol was quantified with a sterol esterase colorimetric assay (catalogue no. 352; Sigma Chemical Co, St Louis) by using authentic Centers for Disease Control and Prevention standards (catalogue no. C7921; Sigma Chemical Co). Four standard curves were produced and samples were assayed in triplicate on a Hewlett Packard 8452 spectrophotometer (Hewlett Packard, Wilmington, DE). When plasma samples had been subjected to thawing and refreezing, within- and between-run CVs were $<3\%$ and 5% , respectively. For the violent and nonviolent comparison subjects, an automated colorimetric sterol esterase assay was performed on fresh samples in the clinical laboratories within the Clinical Center of the National Institutes of Health (Hitachi 917; Boehringer Mannheim, Inc, Indianapolis). All samples were collected in heparin. This method was also standardized to authentic Centers for Disease Control and Prevention standards.

Neurotransmitter metabolite assays

Concentrations of the major metabolites of serotonin and dopamine, 5-HIAA and homovanillic acid (HVA), respectively, were quantified in the CSF with HPLC using electrochemical



detection for healthy volunteers and early- and late-onset alcoholics (39). Samples from alcoholics were assayed in one group whereas samples from healthy volunteers were assayed in batches. Within- and between-run CVs were <10%, as reported previously (27, 39). For the violent and nonviolent comparison subjects, CSF 5-HIAA and HVA were quantified by gas chromatography–mass spectroscopy using deuterated internal standards and within- and between-run CVs were <5%, as reported previously (40). The 2 assays yielded comparable results (40).

Statistical analyses

Statistical analyses were performed by using STATISTICA for WINDOWS 1.0 (Statsoft, Tulsa, OK) and STATVIEW 4.1 (Abacus Concepts, Berkeley, CA). Differences between groups were examined by using analyses of variance. In separate analyses, Pearson product-moment correlation coefficients were computed to assess the relations between each CSF monoamine metabolite concentration and age, total cholesterol, liver enzymes, frequency of alcohol consumption, quantity of alcohol consumption, height, weight, body mass index, and Hollingshead social class score. Forward stepwise multiple regression analysis was also computed. Bonferroni corrections for multiple post hoc testing were made throughout, as appropriate.

RESULTS

Plasma total cholesterol did not differ between the groups despite inclusion of groups of early-onset alcoholics and violent subjects, who are at risk for violent and suicidal behavior (Table 1) (1, 10). However, as expected, hostility scores were greater in the violent group and early-onset alcoholics than in the other groups. The violent group had the highest total score on the Buss-Durkee hostility scale and was significantly different from all other groups (Table 2). The Buss-Durkee total score for early-onset alcoholics differed significantly from all other groups except for the late-onset alcoholics. Early-onset alcoholics scored significantly lower in socioeconomic class than the did healthy comparison and violent and nonviolent groups (Table 3). The late-onset alcoholics were significantly older than the early-onset alcoholics and healthy comparison subjects.

Early- and late-onset alcoholics reported significantly higher alcohol consumption than did either the healthy comparison subjects, the violent group, or the nonviolent group (Table 3). The violent group did not differ from the nonviolent group in frequency or quantity of alcohol intake, aspartate transaminase (AST) activity, alanine transaminase (ALT) activity, results of the CAGE questionnaire (33), or the MAST. Likewise, the early- and late-onset alco-

holics did not differ from each other in these measures of alcohol usage or liver damage.

No differences in CSF HVA were found in a 5-group comparison. In a 2-group comparison of healthy comparison subjects and early-onset alcoholics there was no significant difference ($P < 0.02$) after correction for multiple testing (Table 3). In a 2-group comparison of early- and late-onset alcoholics, differences in CSF 5-HIAA ($P < 0.05$) and HVA ($P < 0.07$) were similar to those reported by Fils-Aime et al (27), but with the inclusion of healthy comparison subjects and violent and nonviolent subjects, no differences were found in a 5-group comparison. No significant differences in CSF 5-HIAA remained after correction for multiple testing comparing healthy comparison subjects to the violent group ($P < 0.03$) and comparing the violent with the nonviolent groups ($P < 0.02$). In this study, significant differences in CSF 5-HIAA concentrations were found only between the violent group and the late-onset alcoholics ($P < 0.001$) (Table 4).

Univariate regression results

When all groups were combined ($n = 234$), age was significantly correlated with both plasma total cholesterol ($r = 0.29$, $P < 0.0001$) and CSF 5-HIAA ($r = 0.23$, $P < 0.0005$) concentrations and was thus considered a significant confounding factor in the relation between plasma total cholesterol and CSF 5-HIAA. Height was weakly correlated with CSF 5-HIAA concentration when all groups were combined ($r = 0.14$, $P < 0.05$). Measures of alcohol consumption that included frequency, quantity, MAST scores, CAGE scores, and measures of alcohol-related complications that included ALT and AST assays did not predict CSF 5-HIAA or CSF HVA concentrations in any group or when all groups were combined.

Analysis of predictors of CSF 5-HIAA

In univariate correlational analysis without correction for age, plasma total cholesterol was weakly correlated with CSF 5-HIAA concentrations when all groups were combined ($n = 234$; $r = 0.18$, $P < 0.01$) (Table 4). The independent factors that predicted CSF 5-HIAA at a minimum of $P < 0.05$ (age, height, and plasma total cholesterol) were included in forward stepwise multiple regression analysis models applied to each group. In this multiple regression model, only age and height were selected as predictors of CSF 5-HIAA concentrations when all groups were combined ($\beta = 0.25$, $r^2 = 0.06$, $P < 0.005$). In healthy comparison subjects, age alone was selected as a predictor of CSF 5-HIAA concentrations ($\beta = 0.33$, $r^2 = 0.11$, $P < 0.05$). In forward stepwise multiple regression models, total cholesterol remained

TABLE 1
Plasma cholesterol and neurotransmitter metabolite concentrations¹

	Healthy comparison group	Early-onset alcoholic group	Late-onset alcoholic group	Violent group	Nonviolent group	F value
Total cholesterol (mg/L)	4.56 ± 0.9 ²	4.07 ± 1.3	4.64 ± 1.3	4.43 ± 0.6	4.48 ± 0.1	0.2
CSF HVA (mg/L)	174 ± 72	145 ± 56	169 ± 70	152 ± 71	158 ± 72	1.7
CSF 5-HIAA (mg/L)	91 ± 34	86 ± 31	101 ± 43	73 ± 28 ³	95 ± 34	3.1 ⁴

¹CSF, cerebrospinal fluid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindolacetic acid.

² $\bar{x} \pm SD$.

³Significantly different from late-onset alcoholics, $P < 0.001$ (pairwise ANOVA).

⁴ $P < 0.005$ for overall differences across groups.

TABLE 2

Descriptive variables of study groups

	Healthy comparison group (n = 49)	Early-onset alcoholic group (n = 88)	Late-onset alcoholic group (n = 39)	Violent group (n = 27)	Nonviolent group (n = 31)	P value ¹
Alcohol-dependent subjects	0	88	39	13	13	
Men	38	84	33	22	21	
Women	11	4	6	9	6	
Age (y)	38 ± 16 ^{2,3}	36 ± 9 ⁴	45 ± 8	38 ± 6	40 ± 8	<0.001
Height (cm)	170 ± 20	175 ± 12	173 ± 13	175 ± 10	173 ± 16	NS
Weight (kg)	75 ± 12	78 ± 13	78 ± 13	77 ± 17	80 ± 13	NS
Hollingshead scale	4 ± 1 ⁵	3 ± 1 ^{6,7}	3 ± 1	4 ± 1	4 ± 1	<0.0001
Buss-Durkie hostility score	20 ± 15 ^{5,8}	34 ± 11 ^{6,7}	29 ± 13 ⁸	45 ± 14 ⁷	21 ± 9	<0.0001

¹Overall differences across groups.² $\bar{x} \pm SD$.^{3,4}Significantly different from late-onset alcoholic group (pairwise ANOVA): ³ $P < 0.001$, ⁴ $P < 0.0001$.⁵Significantly different from early-onset alcoholic group, $P < 0.0001$ (pairwise ANOVA).^{6,8}Significantly different from violent group (pairwise ANOVA): ⁶ $P < 0.001$, ⁸ $P < 0.0001$.⁷Significantly different from nonviolent group, $P < 0.0001$ (pairwise ANOVA).

weakly correlated with CSF 5-HIAA concentrations only in the late-onset alcoholics ($\beta = 0.39$, $r^2 = 0.16$, $P < 0.05$). The late-onset alcoholic group did not have a normal distribution of age, CSF 5-HIAA concentrations, or height, which may account for the weakly positive correlation between CSF 5-HIAA and total cholesterol concentrations that did not remain significant after correction for multiple testing.

CSF 5-HIAA and CSF HVA concentrations were significantly intercorrelated ($r = 0.66$, $P < 0.0001$) when all groups were combined, consistent with previous reports (11, 15, 19, 27, 40). Thus, if plasma total cholesterol concentrations were robustly correlated with CSF 5-HIAA, then it should also predict CSF HVA concentrations. However, plasma total cholesterol failed to predict CSF HVA concentrations in any group or when all groups were combined or in any multiple regression model.

DISCUSSION

In this study, no association was found between plasma total cholesterol and CSF 5-HIAA concentrations in 2 groups of sub-

jects predisposed to violent and suicidal behavior, 2 comparison groups, or in healthy comparison subjects. These findings failed to confirm the hypothesis that low total cholesterol concentrations in plasma might predict low concentrations of 5-HIAA in CSF. This relation has been advanced as a mechanism linking cholesterol-lowering therapies with increased mortality from violence and suicide (1, 10) because of the strong association between low concentrations of CSF 5-HIAA and suicidal and violent behaviors. Both drug and dietary therapies prescribed to lower plasma cholesterol can alter tissue concentrations of highly unsaturated fatty acids (41), which are highly concentrated in neuronal tissues. Depletion of highly unsaturated fatty acids from neuronal membranes may have more important biophysical consequences in modulating serotonergic neurotransmission than do alterations in cholesterol concentration.

We tested for a relation between CSF 5-HIAA and plasma total cholesterol concentrations while controlling for potentially confounding factors including age, alcohol consumption, height, and predisposition to violence and suicide. After including age and height in stepwise multiple regression models, no relation

TABLE 3Alcohol-related variables¹

	Healthy comparison group	Early-onset alcoholic group	Late-onset alcoholic group	Violent group	Nonviolent group	F value ²
Frequency of drinking (d/last 180 d of drinking)	32 ± 37 ³	128 ± 60 ^{4,5}	124 ± 64 ⁴	49 ± 62 ⁶	39 ± 57 ⁷	21.1
Quantity of alcohol (g alcohol/d when drinking)	46 ± 55	212 ± 98 ^{4,5}	191 ± 156 ⁴	24 ± 30 ⁶	50 ± 79 ⁷	26.7
ALT ($\mu\text{kat/L}$)	316 ± 133	1000 ± 1283 ⁴	716 ± 533	350 ± 333 ⁷	383 ± 283 ⁷	7.3
AST ($\mu\text{kat/L}$)	0.30 ± 0.1	0.78 ± 0.85 ^{4,5}	0.81 ± 0.08 ⁴	0.30 ± 0.08 ⁶	0.32 ± 0.12 ⁷	8.9
CAGE	0.2 ± 1	4 ± 1 ^{4,5}	3 ± 1 ⁴	1 ± 2 ⁴	1 ± 2 ^{4,7}	82.0
MAST	2 ± 2	41 ± 10 ^{4,5}	35 ± 9 ^{4,5,8}	19 ± 20 ⁴	18 ± 3 ^{4,7}	59.8

¹ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAGE, MAST, Michigan Alcoholism Screening Test (33).²Overall difference across groups, $P < 0.0001$ for all.³ $\bar{x} \pm SD$.⁴Significantly different from healthy comparison group, $P < 0.0001$ (pairwise ANOVA).⁵Significantly different from violent group, $P < 0.0001$ (pairwise ANOVA).⁶Significantly different from late-onset alcoholic group, $P < 0.0001$ (pairwise ANOVA).⁷Significantly different from early-onset alcoholic group, $P < 0.0001$ (pairwise ANOVA).⁸Significantly different from nonviolent group, $P < 0.0001$ (pairwise ANOVA).

TABLE 4

Univariate correlational coefficients (*r*) predicting CSF 5-HIAA by group

	Combined groups	Healthy comparison group	Early-onset alcoholics group	Late-onset alcoholics group	Violent group	Nonviolent group
Age (y)	0.23 ¹	0.33 ²	0.13	0.25	0.27	0.27
Height (cm)	0.14 ²	0.04	0.08	0.31	0.37	0.24
Total cholesterol (mmol/L)	0.18 ²	0.31 ²	0.05	0.39 ²	0.12	0.31

¹*P* < 0.0005.²*P* < 0.05.

was found between plasma total cholesterol and CSF 5-HIAA concentrations when all groups were combined or in any of the subgroups studied. A weak relation remained between plasma total cholesterol and CSF 5-HIAA concentrations in late-onset alcoholics after correction for age and height, but this relation was not significant after correction for multiple testing. No relation between plasma cholesterol and CSF 5-HIAA concentration was found in the violent subjects or early-onset alcoholics, who were the subjects most predisposed to violence or suicide (25). In this study, CSF 5-HIAA and HVA concentrations were significantly intercorrelated, as is the case in healthy volunteers and in most groups of psychiatric patients (11, 15, 19, 27). This finding is probably due to multiple reciprocal interactions between the central serotonergic and dopaminergic systems (42). Thus, the lack of an association between plasma total cholesterol and CSF HVA concentrations in any group adds further confidence to the finding of a lack of association between plasma total cholesterol and CSF 5-HIAA concentrations.

In contrast with reports of lower plasma total cholesterol concentrations in homicidal offenders (28) and in subjects who had medically serious suicide attempts (43, 44), no differences were found in plasma total cholesterol concentrations between the violent subjects and early-onset alcoholics and the other groups. One possible explanation is that homicidal offenders and subjects with medically serious suicide attempts are more impulsive and are a genetically distinct population from the violent and early-onset alcoholic groups. Genetic variants in apolipoproteins or their receptors could account for lower plasma cholesterol concentrations in these groups. Corrigan et al (45) reported that violent prisoners have higher concentrations of apolipoprotein A-IV (*P* < 0.000001) and apolipoprotein E (*P* < 0.0002) than the general population. These apolipoproteins are involved in the transport of both cholesterol and polyunsaturated fatty acids (PUFAs). In HDLs, n-3 fatty acids predicted apolipoprotein E concentrations in the offender group but not in the control group. Corrigan et al (45) also reported low concentrations of plasma docosahexaenoic acid in these violent offenders, which replicated the findings of Virkkunen et al (46), who reported low concentrations of docosahexaenoic acid in violent offenders with antisocial personality disorder. These findings may suggest that abnormalities in plasma cholesterol concentrations and PUFA transportation may be linked by abnormalities in apolipoprotein metabolism.

Endelberg (10) and Muldoon et al (1) proposed that lowering dietary intake of cholesterol, resulting in lowered serum cholesterol, may reduce concentrations of cholesterol in neuronal membranes. Biophysical alterations of membrane properties caused by depleted cholesterol could alter receptor conformation, binding kinetics, or the activity of membrane-bound

enzymes and result in reduced serotonin concentrations in the central nervous system. The biophysical hypothesis put forth by Engelberg (10) seems biologically implausible for total cholesterol but not for highly unsaturated essential fatty acids, in light of the following observations:

- 1) Under normal human dietary conditions, the alteration of dietary cholesterol intake has only a marginal effect on plasma cholesterol concentrations. Intakes of saturated, monounsaturated, and polyunsaturated fatty acids have a much higher correlation with plasma cholesterol concentrations in large epidemiologic studies of human populations than do cholesterol intakes (47).
- 2) Brain cholesterol concentrations are largely unrelated to plasma cholesterol concentrations. A few early studies in the 1950s and 1960s suggested that cholesterol might be able to cross the blood-brain barrier (48), but more recent well-controlled studies have shown that these results were probably artifactual (49). Cholesterol could not be detected crossing into the brain despite control for preparation artifacts and equilibration with endogenous serum preparations (49). Artificial milk that contained either extremely high, low, or normal concentrations of dietary cholesterol profoundly altered plasma and lung cholesterol concentrations, but brain concentrations of cholesterol and sterols were unaltered (50). Edmond et al (50) showed that during development, when the most rapid of accumulation of cholesterol in the brain occurs, the brain is able to synthesize *in situ* all the cholesterol required (50–52). Thus, alterations in dietary intake of cholesterol or in plasma cholesterol concentrations are not likely to influence biophysical properties of neuronal membranes as predicted by Endelberg (10) and Muldoon et al (1). In contrast with cholesterol, the brain is entirely dependent on dietary or maternal sources for essential PUFAs (53) and selectively concentrates docosahexaenoic acid into synaptic neuronal membranes (54). Dietary deficiencies of n-3 essential fatty acids during development result in deficiencies of docosahexaenoic acid in brain tissues (53, 55). In adult brains of cats and rhesus monkeys, alcohol consumption was shown to deplete docosahexaenoic acid (29, 30), which may be an important observation given that more than half of adults committing violent crimes have consumed alcohol chronically (56).
- 3) Although cholesterol is important in determining membrane-order parameters, polyunsaturate composition is a powerful modulator of cholesterol-induced changes in membrane order. In addition to studying cholesterol, Heron et al (57) also showed *in vitro* that linoleic acid, an essential PUFA with 2 double bonds, reduced membrane viscosity and decreased the number of high-affinity sites on serotonin



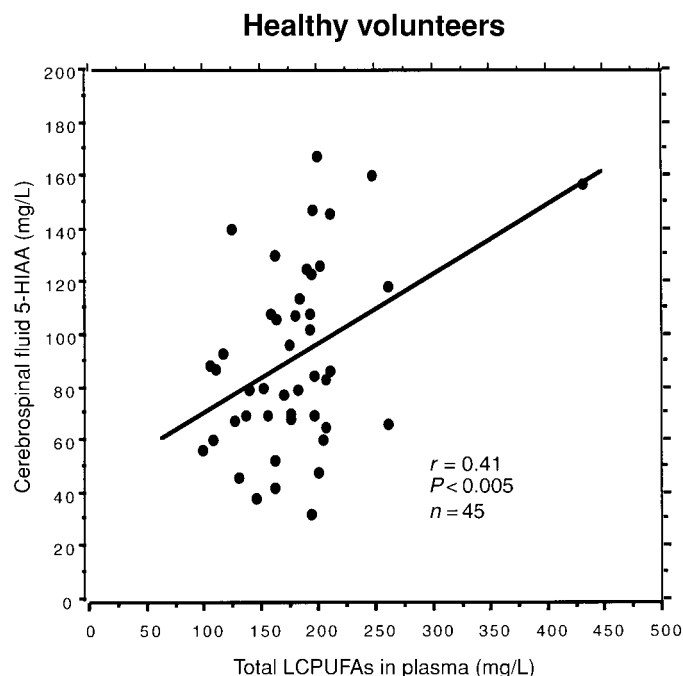



FIGURE 1. Scattergram and simple regression analysis of the relation between plasma total long-chain polyunsaturated fatty acids (20- and 22-carbon fatty acids; LCPUFAs) and cerebrospinal fluid 5-hydroxyindolacetic acid (5-HIAA), a metabolite of serotonin. Regression analysis does not change with exclusion of the outlier. Reproduced with permission from reference 69.

receptors. The receptor rhodopsin and the serotonin receptor are both members of the superfamily of G protein-coupled receptors, sharing both structural and functional similarities. Mitchell et al (58) showed that the membrane phospholipid acyl chain composition was the primary determinant of the relation between bulk membrane packing properties and the formation of metarhodopsin II, the active form of the receptor. Cholesterol was found to have a secondary, modulating effect. In addition, highly unsaturated phospholipids, such as those containing didocosahexaenoate, promoted the formation of metarhodopsin II and markedly diminished cholesterol-induced inhibition of metarhodopsin II formation. Cholesterol-induced membrane condensation, measured by increasing order in hydrocarbon chains, is reduced with greater unsaturation of surrounding acyl chains. Membranes containing docosahexaenoic acid, a highly unsaturated fatty acid, showed the lowest amount of cholesterol-induced condensation (59). Consistent with these findings, Mitchell and Litman (60) showed that cholesterol increases the acyl chain order and slows segmental motion of the acyl chains when measured with the hydrophobic probe diphenyl hexatriene. Membranes containing long-chain PUFAs, especially docosahexaenoic acid, are the least perturbed by the membrane-ordering effects of cholesterol and membranes composed of didocosahexaenoate are virtually unaffected by the addition of cholesterol (60). Highly unsaturated fatty acids, especially docosahexaenoic acid, contribute unique biophysical properties to neuronal membranes, such as lateral domain formation (59–62). These multiple, specific biophysical properties cannot be replicated with cholesterol, monounsaturated, or diunsaturated fatty acids (62, 63), which may contribute to our

understanding of why these specific fatty acids are concentrated in synaptic neuronal membranes (64).

- 4) Animal studies have shown conflicting results when the effects of dietary changes in cholesterol intake on brain serotonin concentrations have been examined. When cholesterol intake was lowered as an isolated dietary variable, rhesus monkeys exhibited more violent behavior, had significantly lower concentrations of CSF 5-HIAA, and had lower serum cholesterol (6.08 mmol/L) than when they consumed a cholesterol-rich diet (16.14 mmol/L) (65). However, in gerbils, no differences in brain tryptophan, serotonin, or 5-HIAA concentrations were found as a function of circulating cholesterol when concentrations were in the range of 1.5–20 mmol/L (66). In contrast, rats maintained on an *n*-3 fatty acid-deficient diet, which reduced concentrations of docosahexaenoic acid in brain tissues, showed a 44% increase in serotonin type 2 receptor number in the frontal cortex (67). This finding was strikingly similar to the report of a 44% increase in serotonin type 2 receptor number in the frontal cortex of suicide victims (68).

Previously, we suggested that plasma total cholesterol may be a surrogate marker for changes in highly unsaturated fatty acid intake or metabolism, which could have a primary role in central serotonin turnover rate (69). In healthy control subjects, we reported (70) that CSF 5-HIAA concentration was robustly correlated with total long-chain PUFAs in plasma (Figure 1), despite correction for age and measures of alcohol intake, which is consistent with this interpretation (69). One point (430 mg long-chain PUFAs/L) could be defined as an outlier because it is >2 SDs from the mean although there was no a priori reason to exclude this subject from analysis. When this point was removed from the regression analysis between plasma long-chain PUFA

and CSF 5-HIAA concentrations, the resulting correlation ($r = 0.307$, $P < 0.04$) was not significantly different in magnitude from the original figure. Total long-chain PUFAs, defined as the sum of 20- and 22-carbon essential fatty acids, also predicted CSF concentrations of HVA, the major metabolite of dopamine ($r = 0.47$, $P < 0.001$). We caution that a causal relation has not yet been shown linking central serotonergic function and dietary or plasma lipids including total cholesterol or highly unsaturated essential fatty acids. The results of 2 recent intervention studies are consistent with this possible causal relation. Piglets were fed 1 of 4 infant formulas for 18 d (71). The formulas were either adequate (C-) or deficient (D-) in 18:2n-6 and 18:3n-3 or contained supplemental arachidonic acid (0.2%, D+) and docosahexaenoic acid (0.16%, C+). Frontal cortex concentrations of serotonin, tryptophan, dopamine, HVA, and norepinephrine were elevated in the D+ and C+ formula groups. Subjects with bipolar affective disorder were treated with 9 g eicosapentaenoic acid + docosahexaenoic acid in a blinded placebo-controlled trial (72). The reductions in manic and depressive episodes were so significant that the trial was stopped after 4 mo. Although plasma total cholesterol concentrations do not predict CSF 5-HIAA concentrations in the populations examined in this study, a relation may yet be found in other more impulsive populations, such as subjects with medically serious suicide attempts. Prospective studies are required to determine the relation between consumption of highly unsaturated fatty acids, cholesterol metabolism, and violent and suicidal behavior or their neurotransmitter metabolites. 

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REFERENCES

- Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. *BMJ* 1990;301:309-14.
- Zureik M, Courbon D, Ducimetiere P. Serum cholesterol concentration and death from suicide in men: Paris prospective study. *BMJ* 1996;313:649-51.
- Gallerani M, Manfredini R, Caracciolo S, Scapoli C, Molinari S, Fersini C. Serum cholesterol concentrations in parasuicide. *BMJ* 1995;310:1632-6.
- Brown LS, Salive ME, Harris TB, Simonsick EM, Guralnik JM, Kohout FJ. Low cholesterol concentrations and severe depressive symptoms in elderly people. *BMJ* 1994;308:1328-32.
- Harrison RWS, Ashton CH. Do cholesterol lowering agents affect brain activity? A comparison of simvastatin, pravastatin, and placebo in healthy volunteers. *Br J Pharmacol* 1994;37:231-6.
- Cummings P, Psaty BM. The association between cholesterol and death. *Ann Intern Med* 1994;120:848-55.
- Wardle J, Armitage J, Collins R, Wallendszus K, Keech A, Lawson A. Randomised placebo controlled trial of effect on mood of lowering cholesterol concentration. The Oxford Cholesterol Study Group. *BMJ* 1996;313:75-8.
- Weidner G, Connor SL, Hollis JF, Connor WE. Improvements in hostility and depression in relation to dietary change and cholesterol lowering. *Ann Intern Med* 1992;117:820-3.
- Hamazaki T, Sawazaki S, Kobayashi M. The effect of docosahexaenoic acid on aggression in young adults. A double-blind study. *J Clin Invest* 1996;97:4:1129-34.
- Endelberg H. Low cholesterol and suicide. *Lancet* 1992;339:727-9.
- Stanley M, Traskman-Bendz L, Dorovini-Zis K. Correlations between aminergic metabolites simultaneously obtained from human CSF and brain. *Life Sci* 1985;37:1279-86.
- Linnoila VM, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK. Low cerebrospinal fluid levels of 5-hydroxyindolacetic acid concentrations differentiate impulsive from non-impulsive violent behavior. *Life Sci* 1983;33:2609-14.
- Roy A, Virkkunen M, Linnoila M. Serotonin in suicide, violence, and alcoholism. In: Coccaro E, Murphy P, eds. *Serotonin in major psychiatric disorders*. Washington, DC: American Psychiatric Association, 1991:187-208.
- Virkkunen M, Rawlings R, Tokola R, et al. CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic violent offenders, fire setters, and healthy volunteers. *Arch Gen Psychiatry* 1994;51:20-7.
- Mann JJ. Violence and aggression. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: the fourth generation of progress*. New York: Raven Press, Ltd, 1995:1919-28.
- Engström G, Alsen M, Regnell G, Traskman-Benz L. Serum lipids in suicide attempters. *Suicide Life Threat Behav* 1995;25:393-400.
- Steegmans PHA, Fekkes D, Hoes AW, van der Does E, Grobbee DE. Low serum cholesterol concentration and serotonin metabolism in men. *BMJ* 1996;312:221.
- Modai I, Valevski A, Kikinson L, Jerushalmy Z, Weizman A. Lack of association between cholesterol blood levels and platelet serotonin uptake. *Eur Psychiatry* 1995;10:352-4.
- Higley JD, Mehlman PT, Poland RE, et al. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. *Biol Psychiatry* 1996;40:1067-82.
- Lerer B, Gillion D, Lichtenberg P, Gorfine M, Gelfin Y, Shapira B. Interrelationship of age, depression and central serotonergic function: evidence from fenfluramine challenge studies. *Int Psychogeriatr* 1996;8:83-102.
- Ganziago JM, Buring JE, Breslow JL, et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N Engl J Med* 1993;329:1829-34.
- Sabesin SM, Bertram PD, Freeman MR. Lipoprotein metabolism in liver disease. *Adv Intern Med* 1980;25:117-46.
- LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: clinical evidence. *Biol Psychiatry* 1994;36:326-37.
- LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: findings in animal studies. *Biol Psychiatry* 1994;36:395-421.
- Buydens-Branchey L, Branchey MH, Noumair D. Age of onset of alcoholism. I. Relationship to psychopathology. *Arch Gen Psychiatry* 1989;46:225-30.
- Roy A, Virkkunen M, Linnoila VM. Reduced serotonin turnover in a subgroup of alcoholics? *Prog Neuropsychopharmacol Biol Psychiatry* 1987;11:173-7.
- Fils-Aime M, Eckardt MJ, George DT, Brown GL, Mefford I, Linnoila VM. Early-onset alcoholics have lower cerebrospinal fluid 5-HIAA than late-onset alcoholics. *Arch Gen Psychiatry* 1996;53:221-16.
- Virkkunen M. Serum cholesterol levels in homicidal offenders: a low cholesterol level is connected with a habitually violent tendency under the influence of alcohol. *Neuropsychobiology* 1983;10:65-9.
- Pawlosky RJ, Salem N Jr. Ethanol exposure causes a decrease in docosahexaenoic acid and an increase in docosapentaenoic acid in feline brains and retinas. *Am J Clin Nutr* 1995;61:1284-9.
- Pawlosky RJ, Salem N Jr. Chronic alcohol exposure in rhesus monkeys alters electroretinograms and decreases levels of neural docosahexaenoic acid. *Alcohol Clin Exp Res* 1999;23:311-7.
- Williams JB, Gibbon M, First MB, et al. The structured clinical interview for DSM-III-R (SCID). II. Multisite test-retest reliability. *Arch Gen Psychiatry* 1992;49:630-6.

32. Endicott J, Spitzer RL. A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* 1978; 35:837-8.
33. Strogaad H, Neilsen SD, Gluud C. The validity of the Michigan alcohol screening test (MAST). *Alcohol Alcohol* 1994;29:493-502.
34. Buss AH, Durkee A. An inventory for different kinds of hostility. *J Consult Psychol* 1957;22:415-8.
35. Eckardt MJ, Parker ES, Noble EP, Feldman DJ, Gottschalk LA. Relationship between neuropsychological performance and alcohol consumption in alcoholics. *Biol Psychiatry* 1978;13: 551-64.
36. Spitzer RL, Endicott J, Robins E. Research diagnostic criteria, rationale and reliability. *Arch Gen Psychiatry* 1978;35:773-82.
37. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, revised. 3rd ed. Washington, DC: American Psychiatric Association, 1987.
38. Muscettola G, Wehr T, Goodwin FK. Effect of diet on urinary MHPG excretion in depressed patients and normal control subjects. *Am J Psychiatry* 1977;134:914-6.
39. Scheinin M, Chang W, Kirk K, Linnoila VM. Simultaneous determination of 3-methoxy-4-hydroxyphenolglycol, 5-hydroxyindolacetic acid and homovanillic acid in cerebrospinal fluid with high performance liquid chromatography using electrochemical detection. *Anal Biochem* 1983;131:246-53.
40. Polinski RJ, Brown RT, Burns RS, Harvey-White J, Kopin IJ. Low lumbar CSF levels of homovanillic acid and 5-hydroxyindolacetic acid in multiple system atrophy with autonomic failure. *J Neurol Neurosurg Psychiatry* 1988;51:914-9.
41. Hibbeln JR, Salem N Jr. Risks of cholesterol-lowering therapies. *Biol Psychiatry* 1996;40:686-7.
42. Agren H, Mefford IN, Rudorfer MV, Linnoila VM, Potter WZ. Interacting neurotransmitter systems: a nonexperimental approach to the 5-HIAA-HVA correlation in human CSF. *Psychiatry Res* 1986;20:175-93.
43. Kunugi H, Takei N, Aoki H, Nanko S. Low serum cholesterol in suicide attempters. *Biol Psychiatry* 1997;1:196-200.
44. Golier JA, Marzuk PM, Leon AC, Weiner C, Tardiff K. Low serum cholesterol level and attempted suicide. *Am J Psychiatry* 1995; 152:419-23.
45. Corrigan FM, Gray RF, Skinner ER, Strathdee A, Horrobin D. Plasma lipoproteins and apolipoproteins in individuals convicted of violent crimes. In: Hillbrand M, Spitz RT, eds. *Lipids, health and human behavior*. Washington, DC: American Psychological Association Press, 1997:167-78.
46. Virkkunen ME, Horrobin DF, Jenkins DK, Manku MS. Plasma phospholipid essential fatty acids and prostaglandins in alcoholic, habitually violent and impulsive offenders. *Biol Psychiatry* 1987; 22:1087-96.
47. Keys A, Anderson J, Grande F. Serum cholesterol changes in response to diet. IV. Particular saturated fatty acids in the diet. *Metabolism* 1965;14:776-86.
48. Dobbing J. The entry of cholesterol into rat brain during development. *J Neurochem* 1963;10:739-42.
49. Pardridge WM, Mietus LJ. Palmitate and cholesterol transport through the blood brain barrier. *J Neurochem* 1980;34: 463-6.
50. Edmond J, Korsak RA, Morro JW, Torok-Both G, Catlin DH. Dietary cholesterol and the origin of cholesterol in the brain of developing rats. *J Nutr* 1991;121:1323-30.
51. Jones JP, Nicholas HJ, Ramsay RB. The biosynthesis of cholesterol and other sterols by brain tissue: distribution in subcellular fractions as a function of time after injection of [2-¹⁴C] mevalonic acid, sodium [2-¹⁴C]acetate and [U-¹⁴C] glucose into 15-day old rats. *J Neurochem* 1975;24:117-21.
52. Jones JP, Nicholas HJ, Ramsay RB. Rate of sterol formation by rat brain glia and neurons in vitro and in vivo. *J Neurochem* 1975; 24:122-6.
53. Salem N Jr. Omega-3 fatty acids: molecular and biochemical aspects. In: Spiller GA, Scala J, eds. *New roles for selective nutrients*. New York: Alan R Liss Inc, 1989:109-228.
54. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes and plasma of rhesus monkeys. *J Lipid Res* 1990;31:237-47.
55. Salem N Jr., Kim HY, Yergey JA. Docosahexaenoic acid: membrane function and metabolism. In: Simopoulos A, Kifer RR, Martin R, eds. *Health effects of polyunsaturated fatty acids in seafoods*. New York: Academic Press, 1986;15:263-317.
56. Miczek KA, Weerts EM, DeBold JF. Alcohol aggression and violence: biobehavioral determinants. In: Martin SE, ed. *Alcohol and interpersonal violence: fostering multidisciplinary perspectives*. Rockville, MD: National Institutes of Health, 1993:83-119. (NIAAA research monograph no. 24.)
57. Heron DS, Shinitzky M, Hershkovitz M, Samuel D. Lipid fluidity modulates the binding of serotonin to mouse brain membranes. *Proc Natl Acad Sci U S A* 1980;77:7463-7.
58. Mitchell DC, Straume M, Litman BJ. Role of sn-2-polyunsaturated phospholipids and control of membrane receptor conformational equilibrium: effects of cholesterol and acyl chain unsaturation on the metarhodopsin I-metarhodopsin II equilibrium. *Biochemistry* 1992;31:662-70.
59. Huster D, Arnold K, Garwrisch K. Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipid mixtures. *Biochemistry* 1998;37:17299-308.
60. Mitchell DC, Litman BJ. Effect of cholesterol on membrane order and dynamics in highly polyunsaturated phospholipid bilayers. *Biophys J* 1998;75:896-908.
61. Litman BJ, Lewis EN, Levin IW. Packing characteristics of highly unsaturated bilayer lipids: Raman spectroscopic studies of multilamellar phosphatidylcholine dispersions. *Biochemistry* 1991;30:2:313-9.
62. Litman BJ, Mitchell DC. A role for phospholipid polyunsaturation in modulating membrane protein function. *Lipids* 1996;31:S193-7.
63. Niebylski CD, Salem N Jr. A calorimetric investigation of a series of mixed-chain polyunsaturated phosphatidylcholines: effect of sn-2 chain length and degree of unsaturation. *Biophys J* 1994; 67:6:2387-93.
64. Salem N Jr, Niebylski CD. The nervous system has an absolute molecular species requirement for proper function. *Mol Membr Biol* 1995;12:1:131-4.
65. Kaplan JR, Shively CA, Fontenot MB, et al. Demonstration of an association among dietary cholesterol, central serotonergic activity, and social behavior in monkeys. *Psychosom Med* 1994;56:479-84.
66. Fernstrom MH, Verrico CD, Ebaugh A, Fernstrom JD. Diet-related changes in serum cholesterol concentrations do not alter tryptophan hydroxylase rate or serotonin concentrations in gerbil brain. *Life Sci* 1996;58:1433-44.
67. DeLion S, Chalon S, Herault J, Guilloteau D, Besnard J, Durand G. Chronic dietary α -linolenic acid deficiency alters dopaminergic and serotonergic neurotransmission in rats. *J Nutr* 1994;124:2466-76.
68. Stanley M, Mann JJ. Increased serotonin-2 binding in frontal cortex of suicide victims. *Lancet* 1983;1:214-6.
69. Hibbeln JR, Salem N Jr. Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. *Am J Clin Nutr* 1995;62:1-9.
70. Hibbeln JR, Umhau JC, George DT, Salem N Jr. Do plasma polyunsaturates predict hostility and depression? *World Rev Nutr Diet* 1997;82:175-86.
71. Owens SP, Innis S. Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotonergic neurotransmitters in frontal cortex caused by a linoleic and α -linolenic acid deficient diet in formula fed piglets. *J Nutr* 1999;129:2088-93.
72. Stoll AL, Severus WE, Freeman MP, et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch Gen Psychiatry* 1999;56:407-12.

