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Lower high-density lipoprotein cholesterol and increased omega-6 polyunsaturated fatty acids in first-degree relatives of bipolar patients

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ABSTRACT

Background. Lower serum high-density lipoprotein cholesterol and increased ratio of omega-6/omega-3 fatty acids have been reported in unipolar and bipolar depressed patients. Changes in cholesterol and fatty acids have been suggested to affect membrane viscosity and consequently serotonergic neurotransmitter expression.

The goal of this study was to investigate whether lower baseline cholesterol and increased omega-6 and lower omega-3 fatty acids are present in healthy first-degree relatives of bipolar patients compared with controls and whether these changes were associated with neuroendocrine responses to an i.v. tryptophan challenge or mood.

Method. Baseline cholesterol, fatty acids and mood were determined in healthy first-degree relatives of patients with bipolar disorders ($N=30$) and healthy matched controls ($N=15$) (parallel-group design). Prolactin and cortisol were measured following tryptophan infusion.

Results. First-degree relatives showed significantly lower plasma high-density lipoprotein cholesterol and increased total omega-6 fatty acids in phospholipids. Lower total omega-3 and higher total omega-6 fatty acids in phospholipids were positively correlated with peak prolactin response to tryptophan. Lower total omega-3 fatty acids in phospholipids and cholesteryl esters were associated with lower mood.

Conclusions. Abnormalities of lower plasma high-density lipoprotein cholesterol and increased total omega-6 fatty acids in phospholipids in these subjects are in agreement with findings in bipolar and major depressed patients. Changes in fatty acids show an association with central serotonergic parameters. It is suggested that these abnormalities in cholesterol and fatty acids may constitute a trait marker for bipolar disorders.

INTRODUCTION

Abnormalities in cholesterol and fatty acids (FAs) have been described in unipolar depressed and manic bipolar patients (Horrobin, 1990; Swartz, 1990; Hibbeln & Salem, 1995; Stoll *et al.* 1999 *a*, *b*). The role of cholesterol and FAs in the pathophysiology of affective disorders has been related to changes in structural components of cell membranes in the brain. A decrease in cholesterol and FAs affects membrane microviscosity and consequently functioning of neurotransmitter systems, such as tryptophan hydroxylase activity, serotonin (5-HT) uptake and monoamine oxidase activity (Engelberg, 1992; Maes *et al.* 1994, 1996; Buydens-Branchey *et al.* 2000; Terao *et al.* 2000). Consequently, 5-HT turnover in the brain may be impaired and

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hence precipitate to depression (Hibbeln *et al.* 1998*a*, *b*). Significant lower fraction of esterified cholesterol, high-density lipoprotein cholesterol (HDL-C) and total cholesterol have now repeatedly been described in depressed patients (Maes *et al.* 1994, 1997*a*, *b*; Olusi & Fido, 1996; Sarchiapone *et al.* 2001). There is evidence for an inverse relationship between plasma cholesterol levels, severity of depression and suicidal behaviour (Morgan *et al.* 1993; Maes *et al.* 1994; Bocchetta *et al.* 2001; Sarchiapone *et al.* 2001). Almeida-Montes *et al.* (2000) did not find a relationship between total cholesterol and completed suicide. In depression, lowered formation of cholesteryl esters may be related to decreased activity of lecithin-cholesterol acyltransferase (LCAT), a key enzyme that reacts preferentially with free cholesterol of the HDL-C particles to form cholesteryl esters (Hunt & Groff, 1990; Subbaiah *et al.* 1990).

In bipolar disorders (BD), both decreased (Swartz, 1990) and increased (Brandrup & Randrup, 1967) plasma cholesterol levels have been described. Manic episodes have been related more to lower cholesterol levels than depressive or mixed episodes (Hibbeln & Salem, 1995).

The omega-6 (e.g. in seed oil) and omega-3 (e.g. in fish oil) FAs are nutritionally essential fatty acids. The parent FAs of these groups are linoleic (18 : 2omega-6) and α-linolenic acid (18 : 3omega-3), which can be converted respectively to arachidonic acid (AA) (C20 : 4omega-6) and docosahexaenoic acid (DHA) (C22 : 6omega-3) (Hunt & Groff, 1990; Shils & Young, 1994). Depressed patients show depletion of omega-3 FAs in red blood cell membranes (i.e. DHA) (Edwards *et al.* 1998; Peet *et al.* 1998), omega-3 fractions in cholesteryl esters and increased C20 : 4omega-6/C20 : 5omega-3 (AA/eicosapentaenoic acid, EPA) ratio in cholesteryl esters and phospholipids (Maes *et al.* 1996). The severity of depression has been associated with lower red blood cell membrane omega-3 FAs and increased AA/EPA ratio in serum phospholipids (Adams *et al.* 1996; Edwards *et al.* 1998). In BD patients omega-3 FAs may be a promising therapy (Kaplan, 1999; Stoll *et al.* 1999*a*, *b*).

It still remains unclear whether these changes in cholesterol and FAs are directly related to the pathogenesis of depression. Possibly abnormalities in cholesterol and FAs may be state markers, resulting from changes in food intake or starvation during depression (Christophe & Vermeulen, 1992). As clinical remission did not alter cholesterol and FAs levels in depressed patients, these abnormalities have also been regarded as trait markers (Peet *et al.* 1998; Maes *et al.* 1999). In order to investigate the hypothesis of a trait marker, these parameters might be investigated in healthy subjects at genetic risk for affective disorders (Sobczak *et al.* 2000*a*). Neuroendocrine responses following challenge with selective 5-HT stimulating agents, e.g. tryptophan (Trp), have been used to assess brain 5-HT responsivity (Riedel *et al.* 2002). A single dose of 50–100 mg/kg i.v. Trp administration causes a significant increase in prolactin (PRL) and cortisol. A blunted neuroendocrine response may than be interpreted as 5-HT receptor desensitization or impaired 5-HT functioning (Sobczak *et al.* 2002*b*). Some, but not all, studies reported blunted responses in BD (Nurnberger *et al.* 1990; Sobczak *et al.* 2002*b*). In this study baseline cholesterol and FAs and their relation to central 5-HT vulnerability was investigated in FH subjects and matched controls. The primary hypothesis of the present study was: healthy first-degree relatives of bipolar patients (FH) have lower HDL-C, total and esterified cholesterol and lower Σ omega-3 FAs and higher Σ omega-6 FAs (in both phospholipids and cholesteryl esters) plasma values compared to healthy controls.

Based on previous findings of lower total, HDL-C, esterified cholesterol and Σ omega-3 FAs and higher Σ omega-6 FAs in depressed and BD patients these seven variables were chosen as primary dependent variables. All other cholesterol and FAs measures were secondary variables and results may be used for formulation of further hypotheses.

METHOD

Subjects

Subjects were relatives of BD patients (FH subjects) and matched healthy controls. Family members were recruited via BD patients receiving treatment at the Department of Psychiatry, University Hospital Maastricht, via the consumer organization for manic-depressive patients and their families, and via

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advertisements in local newspapers. Healthy control subjects were also recruited via newspaper advertisement.

In the FH group, the main inclusion criterion was having at least one first-degree relative with BD. Control subjects were free of any psychopathological family loading. All subjects were interviewed with an abbreviated version of the Family History Research Diagnostic Criteria (FHRDC) to assess FH (Endicott *et al.* 1975). Individual diagnoses (type I or type II BD, according to DSM-IV) of the patients were verified via the patients' own psychiatrist, applying DSM-IV criteria.

Each control subject was matched with two FH subjects with respect to gender, age, body mass index (BMI) and intelligence (IQ). The rationale for the unequal sample sizes is that one control was matched with one relative of a type I and one of a type II BD patient. The major goal of that study was to investigate differences in mood and cognitive effects of Trp in relatives of type I ($N=22$) and type II ($N=8$) patients and controls. These results has been described elsewhere (Sobczak *et al.* 2002*c*, 2003). IQ was estimated using Groninger Intelligence Test (GIT) subtasks (Vocabulary, Mental Rotation, Mental Arithmetic and Word Analogies) (Luteijn, 1966).

Exclusion criteria for all subjects were: (*i*) current or history of use of psychopharmacological medication; (*ii*) lifetime psychiatric disorder including alcohol or drug abuse; (*iii*) current active physical illness; (*iv*) use of any medication known to affect fatty acid and cholesterol metabolism or endocrine functioning; (*v*) subjects consuming a cholesterol or weight-reducing diet; and (*vi*) pregnancy and current use of lactation.

Physical health was assessed by means of a health questionnaire, a standard physical examination by a physician and a urine screening. The urine test (Combur®) included assessment of leukocytes, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin and blood. A Quick View, one-step pregnancy test (Quidel®) was carried out in female subjects to check for unsuspected pregnancy.

A standardized structured psychiatric interview (Mini International Neuropsychiatric Interview; MINI) (Sheehan *et al.* 1994) was taken of all participants to examine the present psychiatric state and to rule out history of Axis I disorders according to DSM-IV criteria. The (17-item) Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), the Young Mania Rating Scale (YMRS) (Young *et al.* 1978) and the depression scale of the SCL-90 (SCL-90-dep) (Arrindell & Ettema, 1986) were used to verify the absence of depressive, manic and general psychiatric symptomatology (by use of cut-off values). The Medical Ethics Committee of the Academic Hospital of Maastricht approved of the study. All participants signed informed consent.

Design and procedure

The study used a parallel-group design. The groups were subjects with (FH) or without (controls) at least one first-degree relative with BD.

To eliminate response bias related to menstrual cycle, all pre-menopausal women were tested in the follicular phase of the menstrual cycle (Menkes *et al.* 1994; Rasgon *et al.* 2000). We did not register daily nutritional intake.

On each test day, the subjects arrived at 9.00 a.m. after an overnight fast. The i.v. canula was inserted immediately in a forearm vein. After 20 min, at 9.30 a.m. (t_{-30}) (all time points t_x refer to $(-)x$ min (before)/after t_0) baseline blood samples were taken for measurement of cholesterol, FAs, PRL and cortisol. Blood samples to assess cholesterol and FAs were taken once, at t_{-30} on the first test day.

Trp or placebo were administered intravenously at a dose of 7·0 g/1000 ml water respectively 1000 ml saline over a 60-min period. Cortisol and PRL were assessed following Trp challenge at t_{60}, t_{75}, t_{90} and t_{105}.

Thirty minutes after collection, serum tubes were centrifuged (3000 rpm for 10 min) and serum was separated and frozen at −80 °C until thawed for analysis. Samples of FH subjects and controls were analysed simultaneously and all samples from multiple tests in the same patient were measured in the same assay.

Cholesterol

Free cholesterol, total cholesterol, HDL-C and triglycerides were assayed using enzymatic-colorimetric methods based on the technique of Allain *et al.* (1974). Low-density lipoprotein cholesterol (LDL-C) was computed as total

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cholesterol − (HDL-C + triglycerides/5). Very low-density lipoprotein cholesterol (VLDL-C) was computed as triglycerides/5. The esterified-C ratio was computed as (1-free cholesterol : total cholesterol)*100. The ratio HDL-C/LDL-C was also computed.

The analytical inter-assay coefficients of variation (CV) were, respectively, as follows: free and total cholesterol, 0·9%, HDL-C, 2·3% and triglycerides, 1·3%.

Fatty acids

Assays of serum FA were carried out as previously described by Maes *et al*. (1999). Serum lipids were extracted (Folch *et al*. 1957) and the phospholipids and cholesteryl esters isolated by thin layer chromatography (Christophe & Matthijs, 1967). Their FAs were converted into methyl esters (Muskiet *et al*. 1983) whose percentage weight composition was determined after separation on a 25 m × 250 μm df Restek 2330 (cyanopropyl) column (initial temperature 150 °C; 1 min isothermal; programmed to 200 °C at 2 °C/min), installed in a Varian Model 3500 gas chromatograph equipped with a flame ionization detector (285 °C) and with a split/splitless injector (275 °C). Split ratio was 1/15. Peak identification was achieved by spiking with authentic standards (Alltech). Peak integration and calculation was performed electronically with a Varian Model 4290 integrator. The results are given as percentage of total FAs in both phospholipids and cholesteryl esters.

The following saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and dimethylaldehydes (dma), were measured in the phospholipid fraction: C14 : 0; C15 : 0; C16 : 0; C16 : 0dma; C16 : 1t; C16 : 1omega-9; C16 : 1omega-7; C17 : 0; C18 : 0; C18 : 0dma; C18 : 1; C18 : 2omega-6; C20 : 0; C18 : 3omega-3; C18 : 4omega-3; C20 : 1; C20 : 2omega-6; C20 : 3omega-9; C20 : 3omega-6; C22 : 0; C20 : 4omega-6; C20 : 5omega-3; C24 : 0; C22 : 4omega-6; C24 : 1; C22 : 5omega-6 (docosapentaenoic acid omega-6; DPAomega-6); C22 : 5omega-3; C22 : 6omega-3 (docosahexaenoic acid; DHA); C25 : 0. The sums (Σ) of the percentages of omega-3 and omega-6 FAs were computed as well as the ratios of omega-6/omega-3; C20 : 4omega-6/C20 : 5omega-3; and C22:5omega-6/C22:6omega-3. In the cholesteryl esters the following FAs were assayed: C14 : 0; C15 : 0; C16 : 0; C16 : 1omega-9; C16 : 1omega-7; C16 : 2omega-6; C17 : 0; C18 : 0; C18 : 1omega-9; C18 : 2omega-6; C18 : 3omega-6; C18 : 3omega-3; C18 : 4omega-3; C20 : 0; C20 : 1; C20 : 2omega-6; C20 : 3omega-6; C20 : 3omega-9; C20 : 4omega-6; C20 :5omega-3; C24 : 1; C22 : 5omega-6; C22 :5omega-3; C22 : 6omega-3; C23 : 0; C24 : 0. The omega-3 and omega-6 FAs and ratios of omega-6/omega-3 and C20 : 4omega-6/C20 : 5omega-3 were computed. The analytical inter-assay CV was 8·5% ($N = 45$).

Cortisol and prolactin

Cortisol and PRL analyses were performed using an Automated Chemiluminescence's System (ACS: centaur) from Bayer, Germany. The analytical inter-assay CV was 1·6% for cortisol and 0·08% for PRL.

Statistical analysis

Arctan transformations were used to reach normality of distribution and to adjust for heterogeneity of variance between study groups (i.e. ratio HDL-C/LDL-C, esterified cholesterol fraction and FAs).

Group mean differences (controls *v*. FH) were assessed by means of analysis of variance (ANOVA). Pearson's product moment and regression analyses were assessed between primary dependent variables of cholesterol and FAs and baseline HDRS, YMRS, the SCL-90-dep (which was first $\log_e$ transformed) and peak prolactin and cortisol responses following Trp (determined as maximal response after Trp, corrected for placebo). The significance of primary dependent variables was set at $\alpha = 0{\cdot}05$ (two-tailed). The Bonferroni method was used to adjust for multiple comparisons of secondary outcome measures and the significance was set at $\alpha = 0{\cdot}001$ (α/k; k = number of dependent variables). Statistical analyses were performed with SPSS 9.0 for Windows.

RESULTS

Demographic data

The subjects were 30 FH (10 men and 20 women) and 15 controls (four men, 11 women), see Table 1. The relatives were children ($N = 20$), parents ($N = 8$) and siblings ($N = 2$). Twenty-one

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Table 1. *Demographic characteristics; bipolar disorder family loading* (1*st*, 2*nd and* 3*rd degree*), *SCL*-90, *Young Mania Rating Scale* (*YMRS*) *and Hamilton Depression Rating Scale* (*HDRS*)

Measure	FH Mean (S.E.)	Controls Mean (S.E.)
Women, *N*	20	11
Men, *N*	10	4
Family members		
Bipolar disorder	1·3	0·0
Age	40±16	38±14
IQ	116±15	116±14
BMI	25·2±3·2	24·4±2·4
SCL-90-dep	18·9±3·9	17·5±2·0
YMRS	0·83±1·9	0·33±0·7
HDRS	1·43±1·8	0·33±0·6

had a first-degree relative with a type I BD diagnosis, and nine subjects were relatives of type II BD patients. Twenty-four FH subjects had one first-degree relative with BD. There were four subjects who had two relatives, one subject had three relatives and one subject had four relatives with BD.

The FH and control groups did not differ significantly with respect to age, body mass index (BMI), intelligence quotient (IQ) and SCL-90-dep, but FH subjects scored significant higher on baseline HDRS ($t=2{\cdot}27$, df$=1{,}39{\cdot}6$, $P<0{\cdot}05$) but this was not clinically relevant.

Of the female subjects, 21 were pre-menopausal and 10 were post-menopausal; 13 women used oral contraceptives. Twelve subjects were smokers; 10 in the FH group and two controls. Values of FAs were missing for two subjects in the FH group.

Table 2 shows the measurements of serum cholesterol. Table 3 shows the FA composition in serum phospholipids and cholesteryl esters of FH subjects and healthy controls.

Primary dependent variables

In FH subjects, HDL-C was significantly lower ($F=7{\cdot}62$, df$=1{,}43$, $P<0{\cdot}05$) compared with controls. Total and esterified-C did not differ between FH and control groups. FH subjects showed significant increased Σ omega-6 ($F=5{\cdot}52$, df$=1{,}41$, $P<0{\cdot}05$) in serum phospholipids but not in cholesteryl esters. In phospholipids and cholesteryl esters Σ omega-3 did not differ significantly between FH and control subjects.

Table 2. *Measurements of serum cholesterol in first-degree relatives of bipolar patients* (*FH*) *and controls, total cholesterol, free cholesterol and esterified cholesterol as % of total cholesterol, triglycerides, high-density lipoprotein cholesterol* (*HDL-C*), *low-density lipoprotein cholesterol* (*LDL-C*), *very-low-density lipoprotein cholesterol* (*VLDL*), *and ratio HDL-C/LDL-C*

	FH Mean (S.E.)	Controls Mean (S.E.)
Total cholesterol, mg/dl	206·27 (55·61)	203·73 (44·45)
Esterified cholesterol, %	72·11 (9·88)	73·78 (1·03)
Free cholesterol, %	27·89 (9·88)	26·22 (1·03)
HDL-C, mg/dl	50·96 (10·86)*	61·07 (12·95)
Triglycerides, mg/dl	114·30 (54·94)	102·33 (51·72)
LDL-C, mg/dl	134·04 (48·78)	122·20 (41·83)
VLDL-C, mg/dl	22·86 (10·99)	20·47 (10·34)
HDL-C/LDL-C	0·45 (0·22)	0·56 (0·22)

* $P<0{\cdot}05$.

Secondary dependent variables

In FH subjects C20:4omega-6 ($F=6{\cdot}03$, df$=1{,}41$, $P<0{\cdot}02$) was higher. Overall concentration of PUFAs in phospholipids ($F=9{\cdot}45$, df$=1{,}41$, $P<0{\cdot}004$) and cholesteryl esters ($F=4{\cdot}22$, df$=1{,}41$, $P<0{\cdot}05$) were higher in FH. In phospholipids C18:3omega-3 fractions ($F=9{\cdot}90$, df$=1{,}41$, $P<0{\cdot}05$) and cholesteryl esters ($F=7{\cdot}44$, df$=1{,}41$, $P<0{\cdot}05$) were lower in FH subjects. MUFAs in phospholipids were lower in FH subjects compared with controls ($F=5{\cdot}60$, df$=1{,}41$, $P<0{\cdot}03$). But all these effects did not remain significant after Bonferroni corrections for multiple comparisons. Other FA and cholesterol measures did not differ *a priori* between FH and controls.

Intercollections of primary dependent variables

Trp significantly increased PRL ($F(1{,}41)=30{\cdot}17$, $P<0{\cdot}01$) and cortisol ($F(1{,}41)=10{\cdot}86$, $P<0{\cdot}01$) release. Hormonal responses to Trp did not differ at baseline and between FH groups and controls (for more detailed results see Sobczak *et al.* 2002*c*). There were significant positive associations between peak PRL response to Trp and lower Σ omega-3 ($r=-0{\cdot}47$, $P<0.01$) and higher Σ omega-6 ($r=0{\cdot}60$, $P<0{\cdot}01$) in phospholipids (see Fig. 1). SCL-90-dep scale correlated significantly with Σ omega-3 FAs in phospholipids ($r=-0{\cdot}40$, $P<0{\cdot}05$) and cholesteryl esters ($r=-0{\cdot}35$, $P<0{\cdot}05$). PRL

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Table 3. *Fatty acid composition (% of total) of plasma phospholipids and cholesteryl esters in first-degree relatives of bipolar patients (FH) and controls*

	Phospholipids		Cholesteryl esters	
Fatty acids	FH Mean (S.E.)	Controls Mean (S.E.)	FH Mean (S.E.)	Controls Mean (S.E.)
ΣSFA	45·92 (1·61)	46·01 (1·78)	13·79 (1·78)	14·33 (1·64)
ΣMUFA	13·00 (1·28)*	14·41 (2·45)	20·88 (2·42)	22·62 (3·63)
ΣPUFA	39·70 (1·52)*	38·00 (2·07)	63·79 (2·81)*	61·70 (4·12)
20 : 3omega-9	0·12 (0·06)	0·13 (0·06)	0·08 (0·17)	0·05 (0·03)
PUFAomega-6				
18 : 2omega-6	20·99 (2·39)	19·80 (4·10)	53·25 (3·94)	52·58 (4·99)
20 : 2omega-6	0·30 (0·09)	0·31 (0·05)	0·06 (0·06)	0·06 (0·01)
20 : 3omega-6	2·64 (0·62)	2·65 (0·82)	0·68 (0·23)	0·69 (0·15)
20 : 4omega-6 (AA)	9·62 (1·48)*	8·53 (1·34)	6·99 (1·63)	5·98 (1·36)
22 : 4omega-6	0·61 (0·12)	0·58 (0·22)	0·00 (0·01)	0·00 (0·01)
22 : 5omega-6 (DPAomega-6)	0·21 (0·10)	0·21 (0·11)	0·04 (0·13)	0·02 (0·01)
Σ omega-6	34·37 (2·12)*	32·09 (3·73)	61·26 (3·29)	59·61 (4·38)
PUFAomega-3				
18 : 3omega-3	0·14 (0·09)*	0·31 (0·29)	0·43 (0·13)*	0·54 (0·13)
18 : 4omega-3	0·10 (0·07)	0·08 (0·02)	0·07 (0·03)	0·07 (0·03)
20 : 5omega-3 (EPA)	0·93 (0·85)	0·99 (0·55)	1·18 (1·70)	0·79 (0·39)
22 : 5omega-3	0·74 (0·22)	0·78 (0·26)	0·02 (0·02)	0·02 (0·02)
22 : 6omega-3 (DHA)	3·30 (0·93)	3·61 (1·97)	0·80 (0·30)	0·66 (0·22)
Σ omega-3	5·21 (1·72)	5·77 (2·16)	2·48 (1·77)	2·08 (0·61)
Ratios				
Σ omega-6/omega-3	7·29 (2·28)	6·22 (2·02)	33·01 (15·98)	31·62 (11·52)
AA/EPA	16·84 (9·93)	12·15 (8·52)	12·90 (8·67)	9·69 (6·77)
DPAomega-6/DHA	0·07 (0·04)	0·06 (0·04)	0·04 (0·09)	0·05 (0·10)

* $P<0{\cdot}05$.

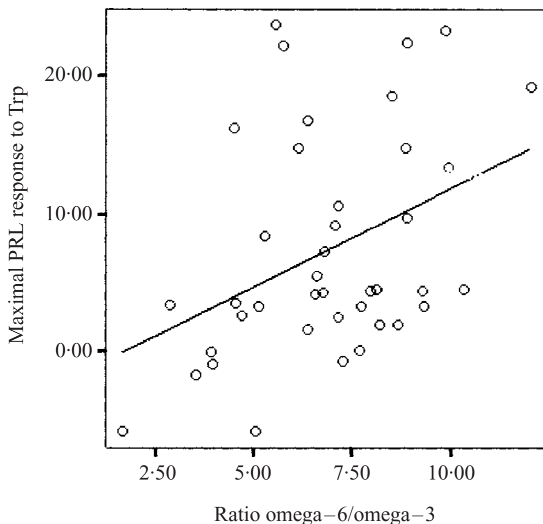

FIG. 1. Positive association between ratio omega-6/omega-3 fatty acids in phospholipids and maximal prolactin (PRL) response (µg/100 ml) to an i.v. tryptophan (Trp) challenge (corrected for placebo). Regression equation: maximal PRL response to Trp = −2·5 + 1·43∗ratio omega-6/omega-3.

response and SCL-90 scales did not correlate significantly with other primary dependent variables.

There were also no significant correlations between primary cholesterol and FAs variables with smoking behaviour, HDRS, YMRS and peak cortisol responses.

DISCUSSION

The first major findings of this study were a significantly lower baseline plasma HDL-C and increased Σ omega-6 FAs in phospholipids in FH subjects compared to controls. Secondly, lower Σ omega-3, higher Σ omega-6 in phospholipids were positively associated with peak PRL response to i.v. Trp challenge. An increase in depressed mood was associated with lower Σ omega-3 FAs in phospholipids and cholesteryl esters.

To our knowledge, this is the first study that investigates cholesterol and FAs in healthy subjects at risk for affective disorders. Lower total cholesterol and HDL-C have also been found in

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depressed patients, which is in agreement with the results of the present study (Maes *et al.* 1997*a*, *b*; Buydens-Branchey *et al.* 2000). High-density lipoprotein plays an important role in transport of cholesterol to the liver. Lower HDL-C may affect adequate cholesterol catabolism. In depression, the combination of lower HDL-C and anti-oxidant activity by vitamin E may result in membrane abnormalities, cellular dysfunctions and also cardiovascular pathology (Booth-Kewley & Friedman, 1987; Maes *et al.* 2000; Bilici *et al.* 2001). Although some (Maes *et al.* 1997*a*, *b*) but not all research data (Maes *et al.* 1994; Hibbeln & Salem, 1995) indicate that low cholesterol may be a state marker in depression, we found no significant correlations between HDL-C or total cholesterol levels and mood in healthy subjects at genetic risk for BD. In BD patients, lower plasma cholesterol levels have been described (Swartz, 1990; Hibbeln & Salem, 1995). As we found low HDL-C in FH subjects who were free of psychopathology, the present findings might provide evidence for low HDL-C to be a trait rather than a state marker in BD. The risk for healthy FH subjects to develop BD is about 15–20%. This suggests that of our research population only six to eight subjects actually have this trait. It is, therefore, premature to point out abnormal cholesterol status as a definite trait marker for BD in all subjects with a positive family history. However, present findings implicate the contribution of HDL-C in a vulnerability to develop BD. This vulnerability is also determined by other genetic and environmental risk factors. Cohort investigation of this research population might detect subjects prone to develop BD and might further elucidate the potential of lower HDL-C as a trait marker.

Next, Σ omega-6 FAs were higher in phospholipids in FH subjects compared to controls. Higher Σ omega-6 FAs were primary due to higher plasma C20:4omega-6. Though not significant there was a tendency towards lower omega-3 FAs i.e. C18:3omega-3. Independent of FH, lower Σ omega-3 FAs were correlated with a more depressed mood. This finding of higher omega-6 is in agreement with findings in BD and depressed patients and may again suggest evidence for a trait marker. The role of omega-3 FAs in establishing mood has also been shown in BD patients in which omega-3 FAs supplementation has mood stabilizing effects. This effect of omega-3 FAs on mood might be ascribed to stabilization of the 5-HT system and its anti-kindling properties (Sullivan *et al.* 1994; Kaplan, 1999; Stoll *et al.* 1999*a*, *b*). Repletion of omega-3 FAs in BD and FH subjects may therefore influence membrane functioning and signal transduction in the brain resulting in normalization of mood (Sullivan *et al.* 1994; Stoll *et al.* 1999*a*, *b*). The literature on FAs in BD is limited, but the abnormalities in FAs as described in FH subjects are in agreement with findings in major depression. In depressed patients, lower omega-3, higher omega-6 FAs and higher ratio omega-6/omega-3 have been described (Adams *et al.* 1996; Maes *et al.* 1996, 1999; Edwards *et al.* 1998; Seko, 1999).

The present study showed no changes in esterified cholesterol in FH subjects, which might suggest no changes in LCAT activity. The results of Maes *et al.* (1994, 1999) suggested decreased esterified cholesterol in depressed patients, which might be related to increased pro-inflammatory cytokines in depression. As FH subjects were healthy and free of any psychopathology, acute activation of the inflammatory response system was not expected, which may explain the absence of changes in esterified cholesterol in FH subjects.

In the second part of the study, the association between baseline cholesterol and FAs with hormonal responses to an i.v. Trp challenge was investigated. Lower Σ omega-3 and higher Σ omega-6 FAs in all subjects were accompanied by enhanced PRL response following i.v. Trp challenge. We assume that an enhanced neuroendocrine response is the result of 5-HT receptor sensitization or elevated brain 5-HT functioning (e.g. second messengers, 5-HT transport). The most likely explanation of these results is an association between abnormalities in FAs and central 5-HT functioning (Riedel *et al.* 2002; Sobczak *et al.* 2002*b*). The current findings are in agreement with a contribution of lower omega-3 and higher omega-6 FAs as described in FH subjects and major depressed patients to central 5-HT dysfunctions, severity of depression and suicidal behaviour (Adams *et al.* 1996; Maes *et al.* 1996; Peet *et al.* 1998). Indeed, deficits in omega-3 FAs have been associated with reduced brain 5-hydroxyindoleacetic acid

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(5-HIAA) concentrations and increased 5-HT receptor sensitization in humans (Hibbeln *et al.* 1998 *a*, *b*). The beneficial effects of treatment with omega-3 FAs in BD and depressed patients may thus be obtained by an improvement in 5-HT functioning (Sullivan *et al.* 1994; Stoll *et al.* 1999 *a*, *b*; Mirnikjoo *et al.* 2001). Addition of omega-3 FAs (i.e. EPA) to medication treatment has been shown to be a successful therapy in treatment-resistant depression (Nemets *et al.* 2002; Puri *et al.* 2002).

In depressed patients, suicide and central 5-HT dysfunctions have also been related to low HDL-C and total cholesterol (Engelberg, 1992; Weidner *et al.* 1992; Buydens-Branchey *et al.* 2000; Rabe-Jablonska & Poprawska, 2000; Bocchetta *et al.* 2001; Sarchiapone *et al.* 2001). The findings of the present study provide no evidence for an association between cholesterol and central 5-HT vulnerability. In previous studies, no significant correlations between serum cholesterol levels and brain 5-HIAA levels or D-fenfluramine-induced PRL and cortisol response were shown in depressed patients and healthy controls (Hibbeln *et al.* 1998 *a*; Sarchiapone *et al.* 2001). Hence, Buydens-Branchey *et al.* (2000) and Terao *et al.* (2000) found a significant negative association between lower HDL-C, serum total cholesterol and neuroendocrine responses to oral meta-chlorophenylpiperazine (m-CPP).

Potential confounders were not expected to explain abnormalities in cholesterol and FAs in FH subjects. First, FH subjects were successfully matched to controls with respect to age, gender and BMI. Secondly, all subjects were screened for no cholesterol-restricted or weight-reducing diet. In addition, there were no abnormalities in C20 : 3omega-9 (Mead acid) in any of the subjects, suggesting that a dietary restriction in LA, LNA, omega-3 or omega-6 FAs was not present (Christophe & Vermeulen, 1992). Fatty acid abnormalities in depression have been described in a population from different genetic background and with a different diet (Seko *et al.* 1999). Thirdly, statistical analyses revealed no significant correlation between smoking behaviour and cholesterol and FAs.

In conclusion, the findings of the present study showed abnormalities in baseline HDL-C and omega-6 FAs in FH subjects, which are in agreement with findings in patients with BD and unipolar depression. As FH subjects are at genetic risk for BD but free of psychopathology and concurrent psychotropic medication, abnormalities in cholesterol and FAs provide some evidence for a trait rather than a state marker in BD. Lower omega-3 and higher omega-6 FAs in our study were related to 5-HT receptor sensitivity and may affect 5-HT vulnerability in healthy subjects.

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