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Lack of an association of depression with n-3 polyunsaturated fatty acids in adipose tissue and serum phospholipids in healthy adults

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Abstract

Studies have shown that depression relates to biomarkers of both short-term and long-term polyunsaturated fatty acid intake. However, it is not known which of these two biomarkers is more closely related to depression. The aim of this study was to examine the relationship of depression with both adipose tissue and serum phospholipid polyunsaturated fatty acids and to assess the importance of each of these two biomarkers in relating to depression. This is a cross-sectional study of healthy adults from the island of Crete. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. Subjects were 394 healthy adults (175 males, 219 females) aged 18–60. The sample consisted of farmers from a number of rural communities of Crete. Fatty acids were determined by gas chromatography in adipose tissue and serum phospholipids. Information about depression was obtained through the Beck Depression Inventory (BDI) and Zung Self-rating Depression Scale (ZSRDS). Adipose tissue alpha-linolenic acid (ALA) (C18:3n-3) was inversely correlated to BDI ($r=-0.17$, $p<0.02$). Multiple linear regression analysis taking into account the possible confounding effect of age, gender, body mass index (BMI), smoking and educational level did not confirm this association. The other polyunsaturated fatty acids in adipose tissue were not related to depression. Serum phospholipid polyunsaturated fatty acids did not correlate with depression. This study did not show that the polyunsaturated fatty acids in the adipose tissue are better predictors of depression than those in serum phospholipids.

Keywords: Psychological; Depression; Human; Adipose tissue; Omega-3 fatty acids; Fish oil; Phospholipids

1. Introduction

As indicated by epidemiological studies, increased fish intake is associated with lower depression prevalence (Hibbeln, 1998; Tanskanen et al., 2001). Depletions in docosahexaenoic acid (C22:6n-3) (DHA) as well as other long-chain n-3 polyunsaturated fatty acids (PUFA) have been reported in depression. Studies implementing biomarkers of n-3 PUFA intake such as plasma, red blood cell membrane, serum cholesteryl ester and phospholipid fatty acids have reported lowered proportions of long-chain n-3 PUFA (Peet et al., 1998; Edwards et al., 1998; Adams et al., 1996; Maes et al., 1996, 1999) and occasionally higher proportions of n-6 PUFA in depressed patients as opposed to healthy controls (Adams et al., 1996; Maes et al., 1996, 1999). However, the particular biomarkers assessed mirror the fatty acids consumed over short time periods, ranging from few days to few weeks at the very most (Glatz et al., 1989; Katan et al., 1997).

By contrast, few studies have implemented biomarkers of long-term intake such as the adipose tissue, a biomarker of long-term or habitual dietary fatty acid intake (1–3 years) (Beynen et al., 1980; Dayton et al., 1966). These studies have indicated inverse relationships between depression and long-term n-3 PUFA intake (Mamalakis et al., 2002, 2004, 2006a,c), thereby confirming the results obtained by studies using biomarkers of

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short-term fatty acid intake. However, it is not known whether long-term fatty acid intake is more strongly related to depression than short-term one, or vice versa. In other words, it is not yet known which of these biomarkers are the strongest predictors of depression and are thus more reliable indicators of the true relation between depression and PUFA intake.

A study that examined the relationship of depression with both serum cholesteryl ester and adipose tissue fatty acids showed that adipose PUFA was a stronger predictor of depression than serum cholesteryl ester ones, thereby indicating that long-term PUFA intake is more strongly related to depression than short-term one (Mamalakis et al., 2006b). However, the results of this study have not been replicated using serum cholesteryl esters or some other biomarker of short-term fatty acid intake such as serum phospholipids. No study has yet examined the relationship of depression with both serum phospholipid and adipose tissue fatty acids simultaneously.

The purpose of the present study is to examine the relationship of depression with both adipose tissue and serum phospholipid PUFA and to assess the relative importance of each of these two biomarkers in relating to depression.

2. Methods

2.1. Subjects

The study was conducted in Greece in the years 2004 and 2005. The study sample consisted of 394 healthy adults (175 males, 219 females) from the island of Crete. Subjects were drawn from the population of 42,000 inhabitants of the area of Messara, a rural area in the south of the county of Iraklion. Subjects were a random sample of all the farmers and/or shepherds of the particular rural area. Subjects were between 18 and 60 years of age and the mean age was 44.7 years. Most of the subjects (90%) were between 18 and 57 years of age. Serum phospholipid fatty acid measures were obtained from 381 subjects, whereas 324 subjects consented to provide adipose tissue biopsy samples. The number of subjects that successfully completed the Beck Depression Inventory (BDI) and the Zung Self-rating Depression Scale (ZSRDS) were 257 and 223 respectively. Of all participants, 194 subjects had complete data on the BDI and the rest of the variables measured, whereas 182 subjects had complete data both on the ZSRDS and the rest of the variables. All subjects were informed about the nature and the purpose of this study and signed a consent form. The ethical committee at the University of Crete had previously approved the protocol of this research. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. Data were collected by a team of trained/experienced personnel. The team consisted of physicians, nurses, dieticians and social workers.

2.2. Depression assessment

Depression level was assessed through the use of the Beck Depression Inventory (BDI) and the Zung Self-rating Depression Scale (ZSRDS) (Beck et al., 1961; Zung, 1965). The particular two self-rating depression scales, have been reported to be valid and reliable depression assessment tools (Beck et al., 1988; Griffin and Kogut, 1988; Biggs et al., 1978; Fountoulakis et al., 2001). The rationale for using both the BDI and the ZSRDS is that although both scales are significantly intercorrelated (Griffin and Kogut, 1988), these scales differ in a number of respects. Specifically, whereas the ZSRDS assesses depressive symptoms experienced over an unidentified time frame (generally), the BDI focuses on the depressive symptoms experienced during the preceding one week. Furthermore, the ZSRDS focuses on the frequency whereas the BDI focuses on the intensity/severity of the depressive symptoms experienced (Giambra, 1977). Finally, factor analytic studies indicate that often, different depression scales including the ZSRDS and the BDI, tap on different aspects or dimensions of depression (Giambra, 1977; Faravelli et al., 1986). This has led to the suggestion that depression assessments should rely on the implementation of multiple depression screening instruments (Shaw et al., 1975).

2.3. Anthropometric measures

Body weight was assayed by a digital scale (Seca) with an accuracy of 100 g. Subjects were weighed without shoes, in their underwear. Standing height was measured without shoes to the nearest 0.5 cm with the use of a commercial stadiometer with the shoulders in relaxed position and arms hanging freely. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).

2.4. Questionnaire data

Subjects were asked about their smoking habits and education. Smoking and educational level were categorical variables (non-smokers = 0, smokers = 1), (primary school = 0, at least secondary school = 1).

2.5. Adipose tissue measures

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan (1985). Samples were taken from the left upper outer quadrant of the gluteal area. Prior to aspiration, aspiration sites were sprayed with local anesthetic (ethyl chloride). Adipose tissue samples were stored in −80 °C. Fatty acid analysis was carried out as previously described (Mamalakis et al., 2006b). The fatty acids have been expressed as % of the total fatty acids present in the chromatogram.

2.6. Serum phospholipid fatty acid measurements

Serum (200 μl) was deproteinated with a mixture of chloroform/methanol (1/1) and the precipitate was removed by centrifugation. After addition of 750 μl of water, the chloroform layer was transferred into another tube and the solvent was removed by evaporation. The dry lipid fraction was dissolved in a small volume of chloroform and applied onto an amino propyl solid-phase column (Bond-Elut NH2 200 mg, Varian Ass.). The

Table 1
Depression, anthropometric and adipose and serum phospholipid polyunsaturated fatty acid measures (mean±standard deviation) in adults from Crete

	Women			Men			Total		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
AGE	219	44.7	10.3	175	44.7	10.3	394	44.7	10.3
BMI	219	29.9	5.5	174	29.5	4.6	393	29.7	5.1
BDI	148	8.2	6.4	109	6.16	6.1	257	7.3	6.3
ZSRDS	122	37.6	8.0	101	32.9	6.9	223	35.4	7.9
Adipose tissue fatty acids									
C18:2n-6	178	10.3	1.7	146	10.1	1.6	324	10.2	1.6
C18:3n-6	178	0.05	0.01	146	0.05	0.01	324	0.04	0.01
C20:2n-6	178	0.14	0.03	146	0.15	0.03	324	0.15	0.03
C20:3n-6	178	0.3	0.08	146	0.2	0.06	324	0.25	0.08
C20:4n-6	178	0.4	0.1	146	0.4	0.1	324	0.4	0.1
C18:3n-3	178	0.6	0.08	146	0.6	0.07	324	0.6	0.08
C20:3n-3	178	0.05	0.02	146	0.05	0.02	324	0.05	0.02
C20:5n-3	178	0.04	0.02	146	0.04	0.02	324	0.04	0.02
C22:5n-3	178	0.1	0.05	146	0.1	0.04	324	0.1	0.05
C22:6n-3	178	0.1	0.06	146	0.1	0.06	324	0.1	0.06
Serum phospholipid fatty acids									
C18:2n-6	212	17.3	2.5	169	17.1	2.2	381	17.2	2.3
C18:3n-6	164	0.1	0.06	152	0.1	0.04	316	0.1	0.05
C20:3n-6	212	3.2	0.7	169	3.3	0.7	381	3.3	0.7
C20:4n-6	212	9.9	1.8	169	10.2	2.0	381	10.0	1.9
C18:3n-3	192	0.12	0.04	144	0.12	0.05	336	0.1	0.05
C20:5n-3	212	0.7	0.5	169	0.7	0.5	381	0.7	0.5
C22:5n-3	211	0.6	0.1	169	0.6	0.1	380	0.6	0.1
C22:6n-3	212	4.0	1.0	169	3.6	1.0	381	3.8	1.0

cholesterol-bound fatty acids were eluted with hexane. The free fatty acids and mono-, di- and triglycerides were eluted with chloroform/methanol/acetic acid (100/2/2). Then the phospholipids fraction was eluted with chloroform/methanol/water (5/10/4). Both the two fractions containing the cholesterol-bound fatty acids and the phospholipids were treated separately according to the following procedure. The solvents were removed by evaporation under nitrogen. Then the bound-fatty acid esters were hydrolyzed and methylated simultaneously with a mixture of 100 μl toluene and 0.5 ml BF3/MeOH for 60 min at 100 °C in a heating block. After cooling, 800 μl distilled water and 800 μl hexane were added. After shaking and settling, the hexane layer (upper layer) containing the FAME was transferred to GC vials and stored at −20 °C until analysis. The FAME was separated on a 100×0.25 mm ID WCOT fused silica capillary column, coated with a 0.25 μm of CP-Slect CB provided by Varian Ass. using a Varian Ass. GC-3900 gas chromatograph equipped with a CP 8400 auto injector. The Galaxie software was used for quantification and identification of peaks. Baseline separation of over 50 FAME peaks was accomplished by means of mixed FAME standards (Sigma). The analytical conditions employed were as follows: volume injected 1 μl, carrier gas nitrogen (1.1 ml/min), injector temperature 250 °C, FID 275 °C, split ratio 1:20 and oven temperature from 185 °C to 245 °C with stepped temperature program: within total run time 57 min. The fatty acids have been expressed as % of the total fatty acids present in the chromatogram.

2.7. Data analysis

Data were analyzed through the use of the SPSS statistical package. The statistical methods used were Spearman correlations, one-way ANOVA and linear multiple regression analysis. Because BDI and ZSRDS were not normally distributed, logarithmic (Natural log) transformation of the particular measures was applied.

Table 2
Crude and multiple linear regression coefficients for adipose tissue ALA and other correlates of depression measured by BDI

Predictor	Crude beta	Multivariate (standardized) beta	*t*-value	*P*-value
Age	0.01	0.17	2.2	0.03
Gender	−0.43	−0.26	−3.2	0.002
BMI	0.006	0.04	0.5	0.59
Smoking	0.21	0.12	1.49	0.14
Educational level	0.01	0.007	0.1	0.92
Adipose tissue ALA	−1.03	−0.10	−1.32	0.19
Constant	1.63		2.5	0.02

Multiple linear regression analysis was carried out with BDI and ZSRDS depression as the dependent variables and age, gender, body mass index (BMI), educational level, smoking and adipose tissue or serum phospholipids fatty acids as the independent variables. Gender, smoking and educational level were dummy variables (males=1, females=0), (non-smokers=0, smokers=1), (primary school=0, at least secondary school=1).

3. Results

Of all participants, 4.6% had mild to severe depression symptoms (BDI scores>=10 and ZSRDS scores>=50) (Pignone et al., 2002). The proportion of subjects that exceeded the BDI cutoff for severe depression (BDI>=30) was 0.4%, whereas none of the participants exceeded the corresponding ZSRDS cutoff (ZSRDS>=76) (Pignone et al., 2002). Of the participants, 61% had attended primary school and 39% have at least secondary school education, while 33% were smokers and 67% were non-smokers. Table 1 depicts means and standard deviations of age, BMI depression and adipose tissue and serum phospholipid PUFA in the two genders and the entire group.

The correlations of adipose tissue fatty acids C18:2n-6, C20:3n-6, C20:4n-6, C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3, total n-6 fatty acids, total n-3 fatty acids and total n-3/n-6 fatty acid ratios with their respective ones in serum phospholipids were (r=0.27, p<0.0005), (r=0.45, p<0.0005), (r=0.32, p<0.0005), (r=0.04, p>0.54), (r=0.47, p<0.0005), (r=0.20, p<0.0005), (r=0.48, p<0.0005), (r=0.34, p<0.0005), (r=0.39, p<0.0005) and (r=0.37, p<0.0005), respectively.

In univariate analysis BDI was only correlated with adipose tissue alpha-linolenic acid C18:3n-3 (ALA) (r=−0.17, p<0.02). BDI did not correlate significantly with C20:4n-6/C20:5n-3, C20:4n-6/C22:6n-3, C20:4n-6/C18:2n-6, C22:6n-3/C18:3n-3, C18:3n-6/C18:2n-6, or total n-3/n-6 fatty acid ratios in either adipose tissue or serum phospholipids. Multiple linear regression analysis with BDI as dependent variable and age, gender, BMI, cigarette smoking, educational level and adipose tissue ALA as independent variables, indicated that 6% of the variability in the log transformed BDI scores was significantly accounted for by age and gender (F=2.97, p<0.008). Beta coefficients showed that the log transformed BDI depression scores were inversely but not significantly related to ALA in adipose tissue (B=−0.10, t=−1.32, P=0.19). Age was positively and gender inversely associated with depression (Table 2).

In univariate analysis ZSRDS correlated with adipose tissue C20:2n-6 (r=−0.16, p<0.03). ZSRDS did not correlate significantly with C20:4n-6/C20:5n-3, C20:4n-6/C22:6n-3, C20:4n-6/C18:2n-6, C22:6n-3/C18:3n-3, C18:3n-6/C18:2n-6, or total n-3/n-6 fatty acid ratios in either adipose tissue or serum phospholipids. The association between ZSRDS and C20:2n-6 was no longer statistically significant taken the confounding effects of age, gender, BMI, cigarette smoking and educational level into account in multivariate analysis.

ALA in serum phospholipids was not related to depression measured by either BDI or ZSRDS in univariate and multivariate analysis. Also other serum phospholipid polyunsatured fatty acids were not related to depression.

The more depressed sub-sample (BDI or ZSRDS>83th percentile) did not differ significantly in any adipose tissue or serum phospholipid n-3 fatty acid or total n-3 fatty acids than their less depressed counterpart (BDI or ZSRDS<17th percentile). In addition, the two sub-samples did not differ significantly in C20:4n-6/C20:5n-3, C20:4n-6/C22:6n-3, C20:4n-6/C18:2n-6, C22:6n-3/C18:3n-3, C18:3n-6/C18:2n-6, or total n-3/n-6 fatty acid ratios in either adipose tissue or serum phospholipids.

Adipose tissue ALA correlated to BDI in females (r=−0.26, P<0.004) but not in males. This association was no longer statistically significant after controlling for age, BMI, cigarette smoking and educational level in multivariate analysis. There were no significant correlations between depression and other adipose tissue or serum phospholipid fatty acids in either gender.

4. Discussion

The present study failed to replicate findings of previous studies indicating significant inverse relationships between depression and adipose n-3 PUFA in adolescent, adult and elderly subjects (Mamalakis et al., 2002, 2004, 2006a,c). Although there was a significant zero-order correlation between BDI and adipose tissue ALA, this relation was not significant in a multivariate model including age, gender, BMI, cigarette smoking and educational level as covariates. Other adipose tissue polyunsatured fatty acids and serum phospholipid polyunsatured fatty acids were not related to depression. It has been reported that the traditional diet of Crete is characterized by a n-6/n-3 ratio of about 1:1 (Simopoulos, 1999). By contrast, it is estimated that the particular ratio is about 10–20:1 in Northern Europe and the United States (Manios et al., 2006). In the present study, this ratio was 10:1 for adipose tissue and 6:1 for serum phospholipid fatty acids. It may be possible that had the current study been carried out on a Northern European or US population where the particular ratio can exceed that observed in our adults, different results might have been obtained.

In three previous studies individual n-3 PUFA in adipose tissue were inversely related to depression (Mamalakis et al., 2002, 2006a,c). In the present study ALA in adipose tissue was inversely related to BDI in univariate but not in multivariate analysis. Taken together, these studies indicate that a small portion of the variability of depression (adjusted R^2<7%) is significantly accounted for by different n-3 fatty acids in adipose tissue (Mamalakis et al., 2002, 2004, 2006a,c). The results of the studies that have used adipose tissue fatty acid measures, an index of habitual or long-term fatty acid intake, suggest that a small, albeit statistically significant proportion of the variability in depression, is accounted for by long-term or habitual n-3 PUFA intake.

The results of this study indicate that polyunsatured fatty acids in adipose tissue were not stronger related to depression than those in serum phospholipids. Unlike adipose tissue, a biomarker of long-term (1–3 years) fatty acid intake (Beynen et al., 1980; Dayton et al., 1966), serum phospholipids are a biomarker of short-term intake of dietary fatty acids (1–2 weeks) (Katan et al., 1997). This is the first study that has investigated both serum

phospholipid and adipose tissue fatty acids in relation to depression. The results of the present study do not confirm the results of a previous study which indicated that adipose tissue fatty acids were stronger predictors of depression than serum cholesteryl ester fatty acids, another biomarker of short-term fatty acid intake (Mamalakis et al., 2006b).

The observed inverse relationship between gender and depression, in the present study is in congruence with other studies observing higher depression rates in women as opposed to men (Kuehner, 2003). The observed positive relationship between age and depression is in line with findings from other studies (Snowdon, 2001). Unlike other studies, however, this study failed to demonstrate the reported significant relations of depression with BMI (Roberts et al., 2003), smoking (Paperwalla et al., 2004), or educational level (Gallo et al., 1993).

In conclusion, the observed inverse correlation between ALA in adipose tissue and depression did not persist in a multivariate analysis adjusting for age, gender, BMI, cigarette smoking and educational level. Serum phospholipid polyunsatured fatty acids were also not related to depression. Polyunsatured fatty acids in adipose tissue were not better predictors of depression than those in serum phospholipids.

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