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ORIGINAL ARTICLE

Depression and long chain n-3 fatty acids in adipose tissue in adults from Crete

G Mamalakis[1], N Kalogeropoulos[2], N Andrikopoulos[2], C Hatzis[1], D Kromhout[3], J Moschandreas[1] and A Kafatos[1]

[1]*Department of Social Medicine Preventive Medicine and Nutrition, School of Medicine, University of Crete, Iraklion, Crete, Greece;* [2]*Laboratory of Food Chemistry-Biochemistry-Physical Chemistry, Department of Science of Dietetics-Nutrition, Harokopio University, Kallithea, Athens, Greece and* [3]*National Institute for Public Health and the Environment, Nutrition and Consumer Safety Division, Bilthoven, The Netherlands*

Background: Only one study has investigated the relationship of essential fatty acids in the adipose tissue with depression in adults and suggested an inverse relationship between docosahexaenoic acid (22:6 n-3) (DHA) and depression.
Objective: To examine the relation between adipose tissue polyunsaturated fatty acids especially n-3 and n-6 fatty acids, an index of long-term or habitual fatty acid intake, and depression in adults.
Design: Cross-sectional study of healthy adults from the island of Crete.
Setting: The Preventive Medicine and Nutrition Clinic, University of Crete, Greece.
Subjects: A total of 130 healthy adults (59 males, 71 females) aged 22—58 years. The sample was a sub-sample of the Greek ApoEurope study group.
Methods: Fatty acids were determined by gas chromatography in adipose tissue. Information about depression was obtained through the Zung Self-rating Depression Scale.
Results: Adipose tissue DHA was inversely related with depression. Multiple linear regression analysis taking into account the possible confounding effect of age, gender, body mass index, smoking and educational level confirmed this association.
Conclusions: The inverse relationship between adipose DHA and depression in adults, replicates findings of a previous study. This relationship indicates that a low long-term dietary intake of DHA is associated with an increased risk for depression in adults.
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Introduction

Depression constitutes the most common psychiatric disorder in adults and a major health problem in the elderly (Zheng *et al*., 1997; Forsell and Winblad, 1999). It has been reported that the age of onset of major depression has decreased, while its incidence has increased, the last 100 years (Klerman and Weissman, 1989). Depression is associated with increases in all-causes mortality, particularly in men (Zheng *et al*., 1997).

Epidemiological studies have shown that increased consumption of fish is associated with a lower prevalence of depression (Hibbeln, 1998). There are indications, that depletions in docosahexaenoic acid (C22:6 n-3) (DHA) and other long-chain n-3 polyunsaturated fatty acids (PUFA) may be associated with depression. Lower proportions of

Correspondence: G Mamalakis, Department of Social and Preventive Medicine, School of Medicine, University of Crete, PO Box 2208, Iraklion 71003, Crete, Greece.
E-mail: geor40@yahoo.com
Guarantor: G Mamalakis.
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long-chain n-3 PUFA have been reported in the plasma, red blood cell membranes and serum cholesteryl esters and phospholipids of depressed patients as opposed to healthy controls (Adams *et al*., 1996; Maes *et al*., 1996, 1999; Edwards *et al*., 1998; Peet *et al*., 1998). However, not only n-3 PUFA, but also PUFA of the n-6 family were implicated in depression. Elevated ratios of n-6/n-3 PUFA and of arachidonic (C20:4 n-6) to eicosapentaenoic acid (C20:5 n-3) have been observed in erythocytes and serum cholesteryl esters and phospholipids of depressed patients as opposed to healthy controls (Adams *et al*., 1996; Maes *et al*., 1996, 1999). It has been reported that plasma and serum phospholipids and cholesteryl esters reflect fatty acid intake over a few days to weeks (Glatzz *et al*., 1989; Katan *et al*., 1997). However, it has been shown that lecithin:cholesterol acyl transferase (LCAT), the enzyme responsible for fatty acid esterification to cholesteryl esters, has a preference for linoleic acid over n-3 PUFA, especially DHA (Parks *et al*., 1989; Thornburg *et al*., 1995). Indeed, DHA has been reported to be a poor substrate for LCAT (Subbaiah *et al*., 1993). Nevertheless, taken together, these findings appear to indicate that the lower n-3 PUFA in depressed persons reported by most of the studies may reflect, at least in part, a corresponding lower consumption of these particular fatty acids. Controlled clinical studies have shown that dietary supplementation with n-3 PUFA over a short period, led to improvements in depressive symptoms in depressed patients (Nemets *et al*., 2002; Peet and Horrobin, 2002; Su *et al*., 2003).

Few studies have examined the relationship between long-term n-3 PUFA intake and depression. The adipose tissue composition is a biomarker of long-term or habitual dietary fat intake (1–3 years) (Dayton *et al*., 1966; Beynen *et al*., 1980). Three studies have examined the relationship between adipose tissue PUFA and depression. One of these studies indicated an inverse relationship between adipose tissue alpha-linolenic acid (C18:3 n-3) and depression, in a group of elderly (Mamalakis *et al*., 2004a). Depressed subjects had significantly lower (−10.5%) adipose tissue C18:3 n-3 levels than non-depressed subjects. A second study failed to observe a relation between adipose tissue n-3 PUFA and depression in an adolescent group (Mamalakis *et al*., 2004b). It must be emphasized that only one study has examined the relationship between adipose n-3 PUFA and depression in adults. Furthermore, the particular adult group was a homogeneous one in terms of education and occupation (i.e. lawyers). This study indicated that adipose tissue DHA related inversely to depression in the particular adult group (Mamalakis *et al*., 2002). Mildly depressed subjects had 36.4% lower adipose tissue DHA levels than non-depressed subjects.

The purpose of the present study is to re-examine and confirm the findings obtained on depression and adipose tissue n-3 PUFA in adults, this time using a non homogeneous study sample.

Methods

Subjects

The study sample was a sub-sample of the Greek ApoEurope study group (Schiele *et al*., 2000). The sample consisted of 130 healthy adults (59 males, 71 females) from the island of Crete. Subjects were between 22 and 58 years of age. The mean age was 36.9 years. All subjects were informed about the nature and the purpose of this study and signed an informed consent. The ethics committee at the University of Crete had previously approved the protocol of this research. Subjects were interviewed by appointment at the Preventive Medicine and Nutrition Clinic of the University of Crete where they underwent a thorough physical examination and clinical test.

Depression assessment

The level of depression was assessed through the use of a Greek translation of the Zung Self-rating Depression Scale (ZSDS). ZSDS, a 20-item scale, has been reported to constitute a valid and reliable measure of depression (Fountoulakis *et al*., 2001).

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).

Questionnaire data

Subjects were asked about their smoking habits and education. Smoking was a dichotomous variable (no smoking = 0, occasional or regular smoking = 1). Educational level was coded on an interval scale (primary school = 0, secondary school = 1, post-high school education/vocational-technical training = 2, higher education = 3).

Adipose tissue measures

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan (Beynen and Katan, 1985). The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture (Beynen and Katan, 1985). Buttock adipose tissue samples can be safely stored for up to 1.5 years without changes in the component fatty acids (Beynen and Katan, 1985). Samples were taken from the left upper outer quadrant of the gluteal area, through the use of a 10 ml vaccutaneous tube. Prior to aspiration, aspiration sites were sprayed with local anesthetic (ethyl chloride). Adipose

tissue samples were stored in −80°C. Prior to analysis samples were thawed and the fat was transferred to 10 ml screw-capped tubes by means of Pasteur pipettes and several drops (~0.5 ml) of chloroform: methanol (2:1, v/v). Methyl esters of the fat component fatty acids (FAME) were prepared in the screw-capped vials according to the method described by Metcalfe *et al*. (1966). The FAME were separated on a 50 mx0.22 mm BPX 70 capillary column, coated with a 0.25 mm film of cyanopropyl silicone provided by SGE (Melbourne, Australia), using an Agilent Technologies (former Hewlett-Packard HP, Avondale, PA, USA) HP 6890 gas chromatograph equipped with autosampler and with a MSD-5972 mass selective detector as it was described by Mamalakis *et al*. (2001).

The identification of over 40 FAME peaks was accomplished by means of a standard mixture of 37 FAME purchased from Sigma (Sigma L9405, St Louis, MO, USA) and by reference to NIST mass spectra library. The mixed FAME standard was injected periodically to determine slight changes in retention times, while it furthermore served for the calculation of fatty acid response factors. The calculated response factors were found to range between 0.88 and 1.15 and they were applied to the areas derived from the chromatographic traces.

Statistical methods

Data were analyzed through the use of the SPSS statistical package. Since several of the adipose tissue fatty acids were not normally distributed, the rank-order Spearman's correlation coefficient was used to assess unadjusted relationships between adipose tissue essential fatty acids and Zung depression scores.

Multiple linear regression analysis was carried out with Zung depression as the dependent variable and age, gender, BMI, educational level, smoking and adipose tissue DHA as the independent variables. Gender and cigarette smoking were dummy variables (males = 1, females = 0), (smokers = 1, non-smokers = 0). Education was categorized in four levels (primary school = 0, secondary school = 1, post-high school education = 2, higher education = 3).

Results

Table 1 depicts means and s. d. of depression, anthropometric and adipose tissue fatty acid measures for the two genders. of the females, 21.6% had primary education or less, 25.1% had high school education, 14.5% had post-high school education/vocational training and 38.8% had completed college/university while the corresponding proportions for males were 30.9, 19.5, 9 and 40.6%, respectively. Of females 42.3% were smokers and 33.9% of the males. Females had serum total cholesterol (209.1 mg/dl), triglycerides (77.9 mg/dl), HDL-C (58.9 mg/dl), LDL-C (134.7 mg/dl), systolic blood pressure (119.4) and diastolic blood pressure (76.5), while the corresponding levels for males were (227.6 mg/dl), (138.8 mg/dl), (46.7 mg/dl), (153.1 mg/dl), (125.1 mm Hg) and (84.3 mm Hg).

Table 1 Means and s.d. of depression, anthropometric and adipose tissue fatty acid measures in adults from Crete

	Women			*Men*		
	Mean	*s.d.*	N	*Mean*	*s.d.*	N
Age	36.2	6.7	71	37.7	7.9	59
BMI	24.8	4.5	71	27.7	3.8	56
Depression score	33.9	6.4	71	30.4	6.6	59
C18:2 n-6	12.4	1.9	71	12	2.3	59
C18:3 n-6	0.15	0.11	71	0.13	0.09	59
C20:2 n-6	0.19	0.04	71	0.18	0.04	59
C20:3 n-6	0.19	0.06	71	0.20	0.06	59
C20:4 n-6	0.26	0.09	71	0.31	0.11	59
C22:2 n-6	0.04	0.02	68	0.04	0.02	56
C22:5 n-6	0.03	0.02	67	0.04	0.03	54
C18:3 n-3	0.48	0.08	71	0.50	0.12	59
C18:4 n-3	0.30	0.08	71	0.28	0.09	59
C20:3 n-3	0.04	0.02	68	0.05	0.05	57
C20:5 n-3	0.03	0.01	71	0.04	0.02	59
C22:5 n-3	0.11	0.04	71	0.13	0.04	59
C22:6 n-3	0.09	0.03	71	0.10	0.05	59

Table 2 Spearman's correlations between adipose tissue fatty acids and depression in the adults aged 22–58 years from Crete

Fatty acids	*Zung depression*		
	Males (N = 59)	*Females (N = 71)*	*Total (N = 130)*
C18:2 n-6	0.22	−0.04	0.11
C18:3 n-6	0.10	0.09	0.10
C20:2 n-6	0.08	0.08	0.11
C20:3 n-6	−0.08	0.14	−0.00
C20:4 n-6	−0.02	0.11	−0.03
C22:2 n-6	−0.13	−0.04	−0.05
C22:5 n-6	−0.22	0.02	−0.10
C18:3 n-3	0.08	0.12	0.08
C18:4 n-3	0.09	0.17	0.12
C20:3 n-3	0.09	0.01	0.02
C20:5 n-3	−0.16	0.10	−0.08
C22:5 n-3	−0.25	−0.04	−0.20*
C22:6 n-3	−0.26	−0.12	−0.19*

*$P<0.05$.

Table 2 depicts Spearman correlations between depression and adipose tissue fatty acids in the two genders and the entire sample. The long-chain n-3 fatty acids C22:5 n-3 and C22:6 n-3 were inversely related with depression. Most evidence for an association between long chain n-3 fatty acids and depression is for C22:6 n-3, DHA. Because of the strong correlation between C22:6 n-3 and C22:5 n-3 ($r = +0.84$, $p<0.0005$) we used only DHA in further analysis on the association between n-3 fatty acids and depression. The inverse association between adipose tissue DHA and depression remained after adjustment of potential confounders (i.e. age, gender, BMI, smoking and educational level). Also, gender, BMI

Table 3 Crude and multiple linear regression coefficients for adipose tissue DHA and other correlates of depression

Predictor	*Crude beta*	*Multivariate beta*	t-*value*	P-*value*
Adipose tissue DHA	−36.6	−0.22	−2.7	0.008
Smoking	3.14	0.23	2.8	0.006
BMI	0.41	0.27	3.2	0.002
Gender	−4.07	−0.30	−3.5	0.001
Age	0.03	0.03	0.3	0.72
Educational level	−0.84	−0.11	−1.3	0.21
Constant	25.6		7.4	0.0005

and smoking were significantly related with depression (Table 3).

Discussion

This study was carried out in adults and confirmed the association between the adipose tissue n-3 fatty acid DHA and depression. No association was observed between n-6 fatty acids in adipose tissue and depression. In addition independent effects were observed for gender, BMI and smoking in relation to depression.

The observed inverse relationship between gender and depression (Table 3) agrees with studies indicating consistently higher depression rates in women as opposed to men (Kuehner, 2003). The positive relationship between BMI and depression (Table 3) is in line with findings of other studies (Roberts *et al*., 2003). For example, a prospective study of 2123 middle-aged adults indicated that baseline obesity was associated with elevated risk of depression 5 years after. This finding was independent of depression at baseline (Roberts *et al*., 2003). Indeed, obesity may lead to lower self-esteem and subsequent depression (Sheslow *et al*., 1993). The observed positive relation between smoking and depression also agrees with findings of other studies (Anda *et al*., 1990; Paperwalla *et al*., 2004). In a number of studies, it appears that depression is an antecedent of smoking. Unlike other studies, this study failed to demonstrate a significant relation between depression and age (Snowdon, 2001). Also, the present study failed to replicate the inverse relation between depression and educational level often reported in the literature (Gallo *et al*., 1993).

Given that adipose tissue fatty acid composition is a biomarker of long term (1–3 years) or habitual dietary fat intake (Dayton *et al*., 1966; Beynen *et al*., 1980), the observed inverse relationship between adipose tissue DHA and depression, in the present study, indicates that lower long-term dietary DHA intakes are related to a higher depression risk. This result in our adult sample, replicates the finding of our previous study (Mamalakis *et al*., 2002). Mildly depressed subjects had 36.4% lower adipose tissue DHA levels than non-depressed subjects (Mamalakis *et al*., 2002). The inverse relationship between adipose tissue DHA and depression, in the present study, is in congruence with results of other studies that have shown inverse relationships between consumption of fish and depression (Hibbeln, 1998). Furthermore, the inverse relationship between DHA and depression, supports findings of other studies that detected lower levels in long-chain n-3 PUFA in plasma, red blood cell membranes, and serum cholesteryl esters and phospholipids of depressed patients compared to healthy controls (Adams *et al*., 1996; Maes *et al*., 1996, 1999; Edwards *et al*., 1998; Peet *et al*., 1998). Finally, this finding is in line with findings of controlled clinical studies that have shown beneficial effects of n-3 PUFA administration on depression (Nemets *et al*., 2002; Peet and Horrobin, 2002; Su *et al*., 2003). However, unlike other studies that reported elevations in arachidonic (C20:4 n-6) to eicosapentaenoic acid (C20:5 n-3) ratio in depression (Adams *et al*., 1996; Maes *et al*., 1996, 1999), the present study failed to detect any significant correlation between the particular ratios and depression (Table 2).

There are indications that the brain preferentially incorporates esterified over unesterified fatty acids (Thies *et al*., 1994; Lagarde *et al*., 2001). It has been reported that fatty acids esterified to erythrocyte membrane phospholipids closely reflect those of neuronal membranes (Carlson *et al*., 1986; Babin *et al*., 1993). Nevertheless, adipose tissue fatty acids also may be related to brain fatty acids (Christensen and Hoy, 1997; Valenzuela *et al*., 2004; Taha *et al*., 2005). As a result of hydrolysis of adipose tissue triacylglycerols by hormone-sensitive lipase and adipose triglyceride lipase, free fatty acids enter the circulation (Frayn, 1998; Raclot *et al*., 2001; Zimmermann *et al*., 2004). Non-esterified fatty acids, including DHA, supply cells, tissues, organs and brain with fatty acids (Thies *et al*., 1994; Rapoport *et al*., 2001).

It has been reported that n-3 PUFA can suppress some of the pathophysiological features of depression, such as inflammation and immune reactivity markers. Specifically, *in vitro* studies have shown that EPA and DHA suppress IL-6 production by human endothelial cells (Khalfoun *et al*., 1997). EPA and DHA have been reported to suppress the *in vitro* production of IL-1, IL-2, IL-6, TNF-α and INF-γ by human lymphocytes (Purasiri *et al*., 1997). Human studies have indicated that dietary supplementation with EPA and DHA results in suppression of IL-1, IL-2, IL-6 and TNF-α production by monocytes (Calder, 1997). Given that cytokines such as IL-1, IL-2, IL-6 and TNF-α have been reported to relate positively to depression (Maes *et al*., 1991; Maes, 1995; Hestad *et al*., 2003), the observed inverse relationship between adipose tissue DHA and depression, in the present study, may be due to an inhibiting effect of DHA on the production of the particular cytokines.

Another reason for the inverse relationship between adipose tissue DHA and depression, may involve dopaminergic and serotonergic pathways. It was reported that DHA supplementation was associated with increases in the serotonin and dopamine levels in the rat hippocampus (Li *et al*., 2000). Another study showed that DHA and arachidonic acid feeding prevented a decrease in dopaminergic and serotonergic neurotransmitters in animal frontal

cortex (de la Presa Owens and Innis, 1999). Still, another study indicated that deficiencies in n-3 PUFA were associated with lower dopamine levels in rats (Takeuchi *et al*., 2002). Positive correlations were observed between plasma DHA and cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations, in healthy subjects (Hibbeln *et al*., 1998). CSF 5-HIAA and HVA levels reflect central concentrations of serotonin and dopamine respectively (Hibbeln *et al*., 1998). Given that depression is characterized by reduced dopamine and serotonin levels (Bunney, 1975; Price *et al*., 1990), the observed inverse relationship between adipose tissue DHA and depression, may reflect a stimulatory effect of DHA on serotonin and dopamine synthesis.

Finally, some plausible explanation for the inverse relationship between adipose tissue DHA and depression, may relate to the reported neuroprotection conferred by DHA. Specifically, one of the key pathophysiological features of depression is neuronal atrophy and volume loss in the hippocampus (Sheline *et al*., 1999; Sapolsky, 2000). Dietary deficiency in n-3 PUFA has been reported to result in diminished nerve growth factor levels in rat hippocampus (Ikemoto *et al*., 2000). Another study observed decreases in neuron size in the hippocampus of rats fed DHA-deficient diets (Ahmad *et al*., 2002). Some other animal study reported that DHA protected rat hippocampal cultures from glutamate-induced cytotoxicity (Wang *et al*., 2003). DHA has been credited with neuroprotective and neurotrophic properties by a number of animal studies (Lauritzen *et al*., 2000; Polit *et al*., 2001).

An obvious limitation of this cross-sectional study is that it cannot establish a cause–effect relationship between DHA and depression. Whether the observed relationship between adipose DHA levels and depression in the present study reflects a protective effect of long-term DHA intake on depression or is merely an epiphenomenon of depression is not known. However, double-blind, placebo-controlled clinical trails of n-3 fatty acids in major depression and bipolar disorder have provided indications for a causal link between particular fatty acids, including DHA, and depression (Stoll *et al*., 1999; Nemets *et al*., 2002; Peet and Horrobin, 2002; Su *et al*., 2003). It should be born in mind, that the etiology of depression is still unknown. Nevertheless, the significant reductions in depression as a result of n-3 fatty acid administration in clinical trials indicate that these fatty acids may impinge, directly or indirectly, on the biochemical substratum of depression. Another limitation of the present study is that it consisted of predominantly non-depressed subjects. Studies that examine adipose n-3 fatty acids in relationship to depression have not yet been conducted in depressed persons, and are, therefore, needed.

In conclusion, we observed an inverse relationship between adipose tissue DHA and depression, indicating that a high long-term dietary DHA intake lowers the risk of depression. This is the second report on the relationship between adipose tissue DHA and depression in adults. Given the positive relationship between depression and cytokines, such as IL-1, IL-2, IL-6, INF-γ and INF-α, the inverse relationship between DHA and depression, may be the result of an inhibiting effect of the particular fatty acid on cytokine synthesis. Other plausible reasons for this relationship may involve possible stimulatory effects on serotonergic and dopaminergic systems as well as neuroprotection against hippocampal neuronal atrophy and volume loss.

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References

Adams PB, Lawson S, Sanigorski A, Sinclair AJ (1996). Arachidonic to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids* **31** (Suppl), 157–161.

Ahmad A, Moriguchi T, Salem N (2002). Decrease in neuron size in docosahexaenoic acid-deficient brain. *Pediatr Neurol* **26**, 210–218.

Anda RF, Williamson DF, Escobedo LG, Mast EE, Giovino GA, Remington PL (1990). Depression and the dynamics of smoking. A national perspective. *JAMA* **264**, 1541–1545.

Babin F, Sarda P, Limasset b, Descomps B, Rieu D, Mendy F *et al*. (1993). Nervonic acid in red blood cell sphingomyelin in premature infants: an index of myelin maturation? *Lipids* **28**, 627–630.

Beynen AC, Hermus RJ, Hautvast JG (1980). A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. *Am J Clin Nutr* **33**, 81–85.

Beynen AC, Katan MB (1985). Why do polyunsaturated fatty acids lower serum cholesterol? *Am J Clin Nutr* **42**, 560–563.

Bunney WE (1975). The current status of research in the catecholamine theories of affective disorders. *Psychopharmacol Commun* **6**, 599–609.

Calder PC (1997). n-3 polyunsaturated fatty acids and cytokine production in health and disease. *Ann Nutr Metab* **41**, 203–234.

Carlson SE, Carver JD, House SG (1986). High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. *J Nutr* **116**, 718–725.

Christensen MM, Hoy CE (1997). Early dietary intervention with structured triacylglycerols containing docosahexaenoic acid. Effect on brain, liver, and adipose tissue lipids. *Lipids* **32**, 185–191.

Dayton S, Hashimoto S, Dixon W, Pearce ML (1966). Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. *J Lipids Res* **7**, 103–111.

de la Presa Owens S, Innis SM (1999). Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotoninergic neurotransmitters in frontal cortex caused by a linoleic and alpha-linolenic acid deficient diet in formula-fed piglets. *J Nutr* **129**, 2088–2093.

Edwards R, Peet M, Shay J, Horrobin D (1998). Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord* **48**, 149–155.

Forsell Y, Winblad B (1999). Incidence of major depression in a very elderly population. *Int J Geriatr Psychiatr* **14**, 368–372.

Fountoulakis KN, lacovides A, Samolis S, Kleanthous S, Kaprinis SG, Kaprinis GS *et al*. (2001). Reliability, validity and psychometric

properties of the Greek translation of the zung depression rating scale. *BMC Psychiatr* **1**, 6–11.
Frayn KN (1998). Regulation of fatty acid delivery *in vivo*. *Adv Exp Med Biol* **441**, 171–179.
Gallo JJ, Royall DR, Anthony JC (1993). Risk factors for the onset of depression in middle age and later life. *Soc Psychiatr Psychiatr Epidemiol* **28**, 101–108.
Glatzz JF, Soffers AE, Katan MB (1989). Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid in man. *Am J Clin Nutr* **49**, 269–276.
Hestad KA, Tonseth S, Stoen CD, Ueland T, Aukrust P (2003). Raised plasma levels of tumor necrosis factor alpha in patients with depression: normalization during electroconvulsive therapy. *J ECT* **19**, 183–188.
Hibbeln JR (1998). Fish consumption and major depression. *Lancet* **351**, 1213.
Hibbeln JR, Linnoila M, Umhau JC, Rawlings R, George DT, Salem Jr N (1998). Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects, and early- and late-onset alcoholics. *Biol Psychiatr* **44**, 235–242.
Ikemoto A, Nitta A, Furukawa S, Ohishi M, Nakamura A, Fujii Y *et al*. (2000). Dietary n-3 fatty acid deficiency decreases nerve growth factor content in rat hippocampus. *Neurosci Lett* **285**, 99–102.
Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M (1997). Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* **38**, 2012–2022.
Khalfoun B, Thibault F, Watier H, Bardos P, Lebranchu Y (1997). Docosahexaenoic and eicosapentaenoic acids inhibit *in vitro* human endothelial cell production of interleukin-6. *Adv Exp Med Biol* **400**, 589–597.
Klerman GL, Weissman MM (1989). Increasing rates of depression. *JAMA* **261**, 2229–2235.
Kuehner C (2003). Gender differences in unipolar depression: an update of epidemiological findings and possible explanations. *Acta Psychiatr Scand* **108**, 163–174.
Lagarde M, Bernond N, Brossard N, Lemaitre-Delaunay D, Thies F, Croset M *et al*. (2001). Lysophosphatidylcholine as a preferred carrier form of docosahexaenoic acid to the brain. *J Mol Neurosci* **16**, 201–204.
Lauritzen I, Blondeau N, Heurteaux C, Widmann C, Romey G, Lazdunski M (2000). Polyunsaturated fatty acids are potent neuroprotectors. *EMBOJ* **19**, 1784–1793.
Li H, Liu D, Zhang E (2000). Effect of fish oil supplementation on fatty acid composition and neurotransmitters of growing rats. *Wei Sheng Yan Jiu* **29**, 47–49.
Maes M (1995). Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatr* **19**, 11–38.
Maes M, Bosmans E, Suy E, Vandervorst C, DeJonckheere C, Raus J (1991). Depression-related disturbances in mitogen-induced lymphocyte responses and interleukin-1 beta and soluble interleukin-2 receptor production. *Acta Psychiatr Scand* **84**, 379–386.
Maes M, Christophe A, Delanghe J, Altamura C, Neels H, Meltzer HY (1999). Lowered omega-3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. *Psychiatr Res* **85**, 275–291.
Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H (1996). Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. *J Affect Disord* **38**, 35–46.
Mamalakis G, Kafatos A, Manios Y, Kalogeropoulos N, Andrikopoulos N, Kiriakakis M (2001). dipose fat quality versus quantity: relationships with children's serum lipid levels. *Prev Med* **33**, 525–535.
Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A (2004a). Depression and adipose polyunsaturated fatty acids in the survivors of the Seven Countries Study population of Crete. *Prostagl Leukotr Essent Fatty Acids* **70**, 495–501.
Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A (2004b). Depression and adipose polyunsaturated fatty acids in an adolescent group. *Prostagl Leukotr Essent Fatty Acids* **71**, 289–294.
Mamalakis G, Tornaritis M, Kafatos A (2002). Depression and adipose essential polyunsaturated fatty acids. *Prostagl Leukotr Essent Fatty Acids* **67**, 311–318.
Metcalfe LD, Schmitz AA, Pekka JR (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Ann Chem* **18**, 514–515.
Nemets B, Stahl Z, Belmaker RH (2002). Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *Am J Psychiatr* **159**, 477–479.
Paperwalla KN, Levin TT, Weiner J, Saravay SM (2004). Smoking and depression. *Med Clin North Am* **88**, 1483–1494, x–xi.
Parks JS, Bullock BC, Rudel LL (1989). The reactivity of plasma phospholipids with lecithin: cholesterol acyltransferase is decreased in fish oil-fed monkeys. *J Biol Chem* **264**, 2545–2551.
Peet M, Horrobin DF (2002). A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch Gen Psychiatr* **59**, 913–919.
Peet M, Murphy B, Shay J, Horrobin D (1998). Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatr* **43**, 315–319.
Polit L, Rotstein N, Carri N (2001). Effects of docosahexaenoic acid on retinal development: cellular and molecular aspects. *Lipids* **36**, 927–935.
Price LH, Charney DS, Delgado PL, Heninger GR (1990). Lithium and serotonin function: implications for the serotonin hypothesis of depression. *Psychopharmacology (Berl)* **100**, 3–12.
Purasiri P, Mckechnie A, Heys SD, Eremin O (1997). Modulation *in vitro* of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids. *Immunology* **92**, 166–172.
Raclot T, Holm C, Langin D (2001). A role for hormone-sensitive lipase in the selective mobilization of adipose tissue fatty acids. *Biochim Biophys Acta* **1532**, 88–96.
Rapoport SI, Chang MCJ, Spector AA (2001). Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J Lipid Res* **42**, 678–685.
Roberts RE, Deleger S, Strawbridge WJ, Kaplan GA (2003). Prospective association between obesity and depression: evidence from the Alameda County Study. *Int J Obes Relat Metab Disord* **27**, 514–521.
Sapolsky RM (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biol Psychiatr* **48**, 713–714.
Schiele F, De Bacquer D, Vincent-Viry M, Beisiegel U, Ehnholm C, Evans A *et al*. (2000). Apolipoprotein E serum concentration and polymorphism in six European countries: the ApoEurope Project. *Atherosclerosis* **152**, 475–488.
Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci* **19**, 5034–5043.
Sheslow D, Hassink S, Wallace W, DeLancey E (1993). The relationship between self-esteem and depression in obese children. *Ann NY Acad Sci* **699**, 289–291.
Snowdon J (2001). Is depression more prevalent in old age? *Aust NZ J Psychiatr* **35**, 782–787.
Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E *et al*. (1999). Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch Gen Psychiatr* **56**, 407–412.
Su KP, Huang SY, Chiu CC, Shen WW (2003). Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur Neuropsychopharmacol* **13**, 267–271.

Subbaiah PV, Kaufman D, Bagdade JD (1993). Incorporation of dietary n-3 fatty acids into molecular species of phosphatidyl choline and cholesteryl ester in normal human plasma. *Am J Clin Nutr* **58**, 360–368.

Taha AY, Ryan MA, Cunnane SC (2005). Despite transient ketosis, the classic high-fat ketogenic diet induces marked changes in fatty acid metabolism in rats. *Metabolism* **54**, 1127–1132.

Takeuchi T, Fukumoto Y, Harada E (2002). Influence of a dietary n-3 fatty acid deficiency on the cerebral catecholamine contents, EEG and learning ability in rat. *Behav Brain Res* **131**, 193–203.

Thies F, Pillon C, Moliere P, Lagarde M, Lecerf J (1994). Preferential incorporation of sn-2 lysoPC DHA over unesterified DHA in the young rat brain. *Am J Physiol* **267** (5 Part 2), R1273–1279.

Thornburg JT, Parks JS, Rudel LL (1995). Dietary fatty acid modification of HDL phospholipid molecular species alters lecithin: cholesterol acyltransferase reactivity in cynomolgus monkeys. *J Lipid Res* **36**, 277–289.

Valenzuela A, Von Bernhardi R, Valenzuela V, Ramirez G, Alarcon R, Sanhueza J *et al*. (2004). Supplementation of female rats with alpha-linolenic acid or docosahexaenoic acid leads to the same omega-6/omega-3 LC-PUFA accretion in mother tissues and in fetal and newborn brains. *Ann Nutr Metab* **48**, 28–35.

Wang X, Zhao X, Mao ZY, Wang XM, Liu ZL (2003). Neuroprotective effect of docosahexaenoic acid on glutamate-induced cytotoxicity in rat hippocampal cultures. *Neuroreport* **14**, 2457–2461.

Zheng D, Macera CA, Croft JB, Giles WH, Davis D, Scott WK (1997). Major depression and all-cause mortality among white adults in the United States. *Ann Epidemiol* **7**, 213–218.

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M *et al*. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* **306**, 1383–1386.