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Depression and adipose and serum cholesteryl ester polyunsaturated fatty acids in the survivors of the seven countries study population of Crete

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Contributors: GM did the statistical analysis, interpretation of results, collection of bibliography and preparation of the manuscript. AK initiated the study and was the overall study coordinator. He contributed to bibliography collection and preparation of the report. MK and GT performed the adipose fatty acid analyses and contributed in the preparation of the report. EJ and HC carried out the serum cholesteryl ester fatty acid analyses and assisted in the preparation of the manuscript.

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Abstract

Background:

Studies have shown that depression relates to biomarkers of both short- and long-term polyunsaturated fatty acid (PUFA) intake. However, it is not known which of these two biomarkers has the closest relationship to depression.

Objective:

To examine the relationship of depression with both adipose tissue and serum cholesteryl ester PUFA and to assess the importance of each of these two biomarkers in relating to depression.

Design:

Cross-sectional study of healthy elderly men from the island of Crete.

Setting:

The Preventive Medicine and Nutrition Clinic, University of Crete, Greece.

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Subjects:

A total of 150 males, aged 80–96 years. The subjects were survivors of the Greek Seven Countries Study group.

Methods:

Fatty acids were determined by gas chromatography in adipose tissue and serum cholesteryl esters. Information about depression was obtained through the use of the short form of the Geriatric Depression Scale (GDS-15).

Results:

Regression analysis showed that depression related positively to age and serum cholesteryl ester arachidonic/docosahexaenoic fatty acid ratio. The only significant unadjusted correlation between depression and serum cholesteryl ester and adipose fatty acids was with adipose alpha-linolenic acid (ALA) (r=-0.31, P<0.01). Depressed males (GDS-15>5) had lower adipose ALA and sum n-3 fatty acids than non-depressed ones. There were no significant differences between depressed and non-depressed males in serum cholesteryl ester fatty acids. When adipose tissue ALA was included as one of the independent measures in the regression model, the observed positive relation between GDS-15 depression and cholesteryl ester arachidonic/docosahexaenoic ratio failed to persist. Instead, there was a negative relationship between GDS-15 depression and adipose tissue ALA.

Conclusions:

It appears that the fatty acids of the adipose tissue are better predictors of depression than those of serum cholesteryl esters. This indicates that depression relates more strongly to long-term than to short-term fatty acid intake. The reason for this may be the reported slow rate of deposition of dietary PUFA to the brain.

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Introduction

Epidemiological studies have shown that increased consumption of fish is associated with decreases in depression prevalence (Nakane *et al.*, 1988; Hibbeln, 1998; Tanskanen *et al.*, 2001). There are indications, that depletions in docosahexaenoic acid (C22:6n-3) (DHA) and other long-chain n-3 polyunsaturated fatty acids (PUFA) may be associated with depression. Lower proportions of 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 polyunsaturates, but also PUFA of the n-6 family were implicated in depression. Elevated n-6/n-3 PUFA and arachidonic (C20:4n-6) to eicosapentaenoic acid (C20:5n-3) ratios 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). Nevertheless, given that plasma and serum phospholipids and cholesteryl esters reflect fatty acid intake over a few-day to few-week period (Glatzz *et al.*, 1989; Katan *et al.*, 1997), the decreased n-3 PUFA in depression reported by the bulk of the studies, appears to reflect, in part, a corresponding reduced consumption in the particular fatty acids. Three studies have examined the relation of depression and long-term n-3 PUFA intake (Mamalakis *et al.*, 2002, 2004, 2004a). 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). Two of the three studies examining the relationship between adipose tissue PUFA and depression reported negative relations between n-3 PUFA and depression (Mamalakis *et al.*, 2002, 2004).

The studies on depression and serum phospholipid and cholesteryl ester and adipose tissue fatty acids indicate that depression relates to both short-term as well as long-term PUFA intake. However, it is not known which of these biomarkers bare the strongest relation with depression and are thus more reliable indicators of the true relation between depression and PUFA intake. It is not known whether long-term PUFA intake is a better predictor of depression than short-term intake, or vice versa. No studies have as yet examined the relationship of depression with both serum cholesteryl ester 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 cholesteryl ester PUFA and to assess the relative importance of each of these two biomarkers in predicting depression.

Methods

Subjects

The study sample consisted of 150 elderly males from the island of Crete. The subjects were survivors of the Greek Seven Countries Study group, the year 2000. Subjects were living in their own homes and were self-reliant (e.g. able to prepare their own food). Subjects came from rural communities and were involved in small farming business. Depression assessments (GDS-15) were made on 124 subjects. Subjects were between 80 and 96 years of age. The mean age was 84 years, while most of the subjects (72%) were between 80 and 85 years of age. Seventy-eight subjects consented to provide adipose tissue biopsy samples. Serum cholesteryl ester fatty acid measures were obtained from 127 subjects. 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. This is the second study on depression and PUFA in the particular elderly group (Mamalakis *et al.*, 2004). The difference between this study from the previous one lies in that serum cholesteryl ester fatty acid measures had not been included in the statistical analysis in the previous study.

Depression assessment

Depression level was assessed through the use of a Greek translation of the short form of the Geriatric Depression Scale (GDS-15). (GDS-15), a 15-item scale, has been reported to constitute a valid and reliable depression measure (Sheikh and Yesavage, 1986; Almeida and Almeida, 1999; Fountoulakis *et al.*, 1999). Geriatric Depression Scale has been standardized in elderly Greeks (Fountoulakis *et al.*, 1999).

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).

Adipose tissue measures

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by 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 with the aid of Pasteur pipettes and several drops (~0.5 ml) of chloroform:methanol (2:1, v/v). Methyl esters of the fat component fatty acids were prepared in the screw-capped vials according to the method described by Metcalfe *et al.* (1966). Briefly, 20–30 mg of fat sample were saponified with 1.0 ml NaOH in methanol and the fatty acid methyl esters (FAME) were prepared with 14% boron trifluoride in methanol following extraction with hexane after washing with saturated NaCl. The hexane (upper layer) containing the FAME was transferred to GC vials and stored at -20°C until analysis. The FAME were separated on a 100 × 0.25 mm^2 Id.SP-2560 fused silica capillary column, coated with a 0.25 μm of cyanopropyl silicone provided by SUPELCO, using a SHIMADZU GC-17A/FID gas chromatograph equipped with an AOC-20I auto injector. The Class-VP chemstation software was used for quantitation 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 helium (1.1 ml/min), injector temperature 250°C, FID 260°C, split ratio 1:4–1:20 (depending on the sample quantity), and oven temperature from 140 to 245°C with stepped temperature program: within total run time 54 min. The fatty acids have been expressed as percent of the total fatty acids present in the chromatogram.

Serum cholesteryl ester fatty acid measures

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 aminopropyl solid-phase column (Bond-Elut NH_2 200 mg, Varian Ass.). The cholesteryl-bound fatty acids were eluted with hexane. The free fatty acids, triglycerides and phospholipids remained bound to the column. The hexane was removed by evaporation under nitrogen. Then the cholesteryl-fatty acid esters were hydrolyzed and methylated simultaneously with a mixture of 100 μl toluene and 0.5 ml BF_3/MeOH for 60 min at 100°C in a heating block. After cooling, 800 μl destilled 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 were separated on a 100 ×0.25 mm^2 ID WCOT fused silica capillary column, coated with a 0.25 μm of CP-Slect CB provided by Varisn Ass., Middelburg, The Netherlands using a Varian Ass. GC-3900 gas chromatograph equipped with an CP 8400 auto injector. The Galaxie software was used for quantitation 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 to 245°C with stepped temperature program: within total run time 57 min. The fatty acids have been expressed as percent of the total fatty acids present in the chromatogram.

Statistical methods

Data were analyzed through the use of the SPSS statistical package. The statistical methods used were one-way ANOVA, Spearman's correlations and linear multiple stepwise Regression analysis.

Results

Table 1 depicts means and s.d. of serum cholesteryl ester and adipose tissue fatty acids for the subjects that consented to provide adipose tissue biopsy samples (n=78) and for which there were serum cholesteryl ester fatty acid measures (n=127).

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Table 1 - Means and s.d. of serum cholesteryl ester and adipose tissue fatty acids in the male survivors of the Seven Countries Study population of Crete.

	Serum cholesteryl ester			*Adipose tissue*		
	N	***Mean***	***s.d.***	**N**	***Mean***	***s.d.***
Fatty acids						
C18:2n-6	127	40.8	3.9	78	7.8	1.03
C18:3n-6	127	1.0	0.35	78	0.05	0.06
C18:3n-3	127	0.41	0.10	78	0.4	0.05
C20:3n-6	127	0.88	0.19	78	0.17	0.06
C20:4n-6	127	6.43	1.28	78	0.31	0.09
C20:5n-3	127	0.85	0.37	78	0.05	0.02
C22:5n-3	127	0.05	0.2	78	0.15	0.04
C22:6n-3	127	0.60	0.19	78	0.14	0.04
Sum n-6 fatty acids	127	49.1	4.03	78	8.4	1.1
Sum n-3 fatty acids	127	1.91	0.53	78	0.86	0.12
n-6/n-3 ratio	127	27.5	7.6	78	9.9	1.5
C14:0	127	0.75	0.29	78	1.43	0.47
C16:0	127	12.7	1.2	78	14.9	2.0
C18:0	127	0.96	0.22	78	2.5	0.71
Saturated fatty acids	127	14.5	1.5	78	18.8	2.7
C14:1	127	0.13	0.16	78	0.17	0.09
C16:1	127	2.44	1.5	78	0.84	0.14
C18:1	127	29.3	3.0	78	62.7	4.0
Monounsaturated fatty acids	127	31.8	3.4	78	63.7	3.9

Spearman's correlation analysis on the subjects with complete data on both serum cholesteryl ester and adipose tissue fatty acids indicated that shorter-chain serum cholesteryl ester PUFA such as C18:2n-6, C18:3n-6 and C18:3n-3 did not correlate significantly with the respective PUFA of the adipose tissue. However, longer-chain serum cholesteryl ester PUFA correlated significantly with the respective PUFA of the adipose tissue. Specifically, the correlations between serum cholesteryl ester C20:3n-6, C20:4n-6, C20:5n-3 and C22:6n-3 with the respective fatty acids of the adipose tissue were (r=+0.41, P<0.001), (r=+0.32, P<0.01), (r=+0.46, P<0.001) and (r=+0.47, P<0.001), respectively. Serum cholesteryl ester C20:4n-6/C22:6n-3 ratio correlated with that of the adipose tissue (r=+0.57, P<0.0005). In addition, serum cholesteryl ester sum n-3 PUFA correlated with those of the adipose tissue (r=+0.29, P<0.05). Saturated adipose tissue C14:0 and C16:0 correlated significantly with the respective fatty acids of serum cholesteryl esters (r=+0.57, P<0.0005) and (r=+0.40, P<0.0005), respectively. Adipose C18:0 did not correlate significantly with the respective serum cholesteryl ester fatty acid. Sum saturated adipose tissue fatty acids correlated significantly with the respective fatty acids of serum cholesteryl esters (r=+0.42, P<0.0005). Of the monounsaturated fatty acids, adipose C14:1 and C18:1 correlated significantly with the respective fatty acids of serum cholesteryl esters (r=+0.28, P<0.02) and (r=+0.39, P<0.0005) respectively. Adipose C16:1 and sum adipose monounsaturated fatty acids did not correlate significantly with the respective serum cholesteryl ester fatty acids.

There was only one significant correlation between an individual fatty acid or a class of fatty acids to depression, namely a negative correlation to adipose tissue C18:3n-3 (alpha-linolenic acid) (r=-0.31, P<0.01) (Figure 1).

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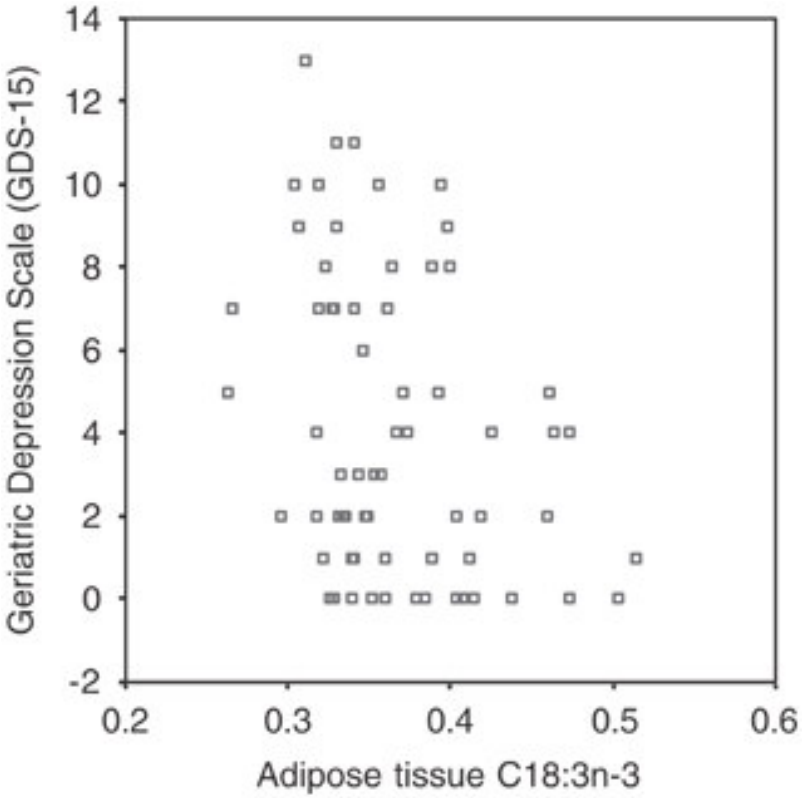

Figure 1. Scatterplot of depression against adipose C18:3n-3.

Table 2 depicts means and s.d. for the serum cholesteryl ester and adipose tissue PUFA in depressed vs non-depressed subjects. Depressed subjects were older than their non-depressed counterpart ($P<0.001$). Depressed subjects had significantly lower adipose tissue alpha-linolenic acid (C18:3n-3) than the non-depressed subjects ($P<0.02$) and this was also reflected in a lower sum of adipose n-3 fatty acids ($P<0.04$). There were no significant differences between the two groups in any other individual fatty acid or groups of fatty acids.

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Table 2 - Means and s.d. of anthropometric, serum cholesteryl ester and adipose tissue fatty acid measures in depressed vs non-depressed survivors of the Seven Countries Study population of Crete.

	Non-depressed (GDS-15≤5)			Depressed (GDS-15>5)			Significance
	N	Mean	s.d.	N	Mean	s.d.	
Adipose tissue fatty acids							
GDS-15	77	2.0	1.6	47	8.6	2.0	$P<0.0005$
Age	77	83.7	3.3	47	86.6	4.2	$P<0.0005$
BMI	64	25.5	4.0	35	25.8	5.5	
C18:2n-6	47	8.0	1.1	21	7.5	0.9	
C18:3n-6	47	0.05	0.01	21	0.07	0.12	
C18:3n-3	47	0.38	0.06	21	0.34	0.03	$P<0.007$
C20:2n-6	47	0.12	0.02	21	0.12	0.02	
C20:3n-6	47	0.17	0.05	21	0.19	0.08	
C20:4n-6	47	0.31	0.08	21	0.31	0.08	
C20:3n-3	47	0.06	0.03	21	0.05	0.02	
C20:5n-3	47	0.05	0.02	21	0.05	0.02	
C22:3n-3	47	0.11	0.04	21	0.11	0.04	
C22:5n-3	47	0.15	0.04	21	0.14	0.04	
C22:6n-3	47	0.15	0.05	20	0.13	0.04	
C20:4n-6/ C22:6n-3	47	2.30	0.81	20	2.60	0.70	
Sum n-6 fatty acids	47	8.6	1.1	21	8.2	1.0	
Sum n-3 fatty acids	47	0.89	0.12	21	0.83	0.10	$P<0.04$
n-6/n-3 ratio	47	9.9	1.6	21	10.0	1.4	
Serum cholesteryl ester fatty acids							
C18:2n-6	66	40.8	4.0	38	40.8	4.0	
C18:3n-6	66	1.0	0.36	38	1.0	0.37	
C18:3n-3	66	0.42	0.10	38	0.39	0.10	
C20:3n-6	66	0.87	0.19	38	0.90	0.20	
C20:4n-6	66	6.30	1.34	38	6.78	1.25	
C20:5n-3	66	0.84	0.35	38	0.87	0.42	
C22:5n-3	66	0.03	0.08	38	0.06	0.12	
C22:6n-3	66	0.58	0.17	38	0.62	0.23	
C20:4n-6/ C22:6n-3	66	11.67	3.81	38	12.67	5.32	
Sum n-6 fatty acids	66	49.02	4.02	38	49.5	4.23	
Sum n-3 fatty acids	66	1.87	0.47	38	1.93	0.61	
n-6/n-3 ratio	66	27.9	7.51	38	27.7	7.7	

Abbreviation: GDS-15=Geriatric Depression Scale.

A multiple linear regression using age, BMI and individual serum cholesteryl ester fatty acids and classes of serum cholesteryl ester fatty acids as independent variables returned a highly significant correlation of depression to age (B=0.29, t=3.0, P<0.003), no correlation to BMI or any individual serum cholesteryl ester fatty acid or classes of fatty acids, and a significant correlation to serum cholesteryl ester C20:4n-6/C22:6n-3 ratio (B=0.20, t=2.1, P<0.04). Together, age and serum cholesteryl ester C20:4n-6/C22:6n-3 ratio significantly accounted for 13% of the variability in GDS-15 depression (F=7.7, P<0.001). As shown by beta coefficients, age is the major predictor of GDS-15 depression followed by serum cholesteryl ester C20:4n-6/C22:6n-3 ratio.

In a subsequent regression model, when adipose tissue C18:3n-3 was also included as one of the independent measures already present in the former regression model, the significant relations of depression to age and cholesteryl ester C20:4n-6/C22:6n-3 ratio failed to persist. Instead, a significant relation between depression and adipose tissue C18:3n-3 emerged. Specifically, 8% of the variability in GDS-15 depression was significantly accounted for by adipose tissue C18:3n-3 (F=6.8, P<0.01). Beta coefficient shows that adipose tissue C18:3n-3 related negatively to depression (B=-0.31, t=-2.6, P<0.01).

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Discussion

It appears that the survivors of the Greek Seven Countries Study group are more depressed than other elderly groups of the same age. For example, 24% of the elderly sample of the Leiden 85-plus study were depressed (GDS-15 $\geq$ 4) (Vinkers *et al.*, 2004). By contrast, 51.6% of the survivors of the Greek Seven Countries Study group were depressed (GDS-15 $\geq$ 4). Also, in a representative study of 14217 people aged 75 years and over in the UK, 7.7% of the 80–84-year-age group were depressed (GDS-15 $\geq$ 6) (Osborn *et al.*, 2003). The corresponding proportion of depression (GDS-15 $\geq$ 6) in the surviving sample of the Greek Seven Countries Study group was 37.9%.

The positive relationship between serum cholesteryl ester C20:4n-6/C22:6n-3 ratio and depression may reflect the opposing effects of C20:4n-6 (AA) and DHA on prostaglandin E-2 (PGE_2) production. AA is the immediate precursor of PGE_2 (Krakauer *et al.*, 1986). On the other hand, DHA inhibits the formation of PGE_2 (Lokesh and Kinsella, 1987; Kelley *et al.*, 1999; Colin *et al.*, 2003). It has been proposed that elevated levels of PGE_2 are implicated in depression (Lieb *et al.*, 1983; Linnoila *et al.*, 1983; Calabrese *et al.*, 1986; Ohishi *et al.*, 1988; Nishino *et al.*, 1989; Colin *et al.*, 2003; Myint and Kim 2003; Muller *et al.*, 2004). Therefore, the elevated serum cholesteryl ester C20:4n-6/C22:6n-3 ratio with increasing depression, in the present study, may be mediated by increases in PGE_2 levels. Unlike other studies that reported reduced levels of EPA in depression, the present study failed to observe a significant inverse relationship between depression and the particular fatty acid (Maes *et al.*, 1996, 1999). Also, this study failed to demonstrate the positive relationships between depression and AA/EPA ratios or sum n-6/sum n-3 PUFA ratios reported by others (Adams *et al.*, 1996; Maes *et al.*, 1996, 1999). This is the first literature report of a relationship between AA/DHA ratio and depression. Clearly, our finding needs to be replicated by other studies.

Another reason for the observed positive relationship between serum cholesteryl ester C20:4n-6/C22:6n-3 ratio and depression may relate to the reported opposing effects of AA and DHA on neuron integrity in the hippocampus. One of the pathophysiological features of depression is neuronal atrophy and volume loss in the hippocampus (Sheline *et al.*, 1999; Sapolsky, 2000). Animal studies have indicated that n-3 PUFA, including DHA, exert neuroprotective effects in the hippocampus (Ikemoto *et al.*, 2000; Ahmad *et al.*, 2002; Wang *et al.*, 2003), whereas AA and its cyclooxygenase and lipogygenase metabolites exert opposite effects (Himmelseher *et al.*, 1996; Paoletti *et al.*, 1998; Kim *et al.*, 2000; Shanker *et al.*, 2004).

The results of this study appear to indicate that adipose fatty acids are stronger predictors of depression than serum cholesteryl ester fatty acids. For example, unlike adipose fatty acids, there were no significant unadjusted correlations between cholesteryl ester fatty acids and depression. Furthermore, although there were significant differences between depressed and non-depressed males in adipose fatty acids, no such differences manifested for cholesteryl ester fatty acids (Table 2). Finally, regression analysis showed that the observed positive relation between GDS-15 depression and cholesteryl ester C20:4n-6/C22:6n-3 ratio failed to persist when adipose tissue C18:3n-3 was included as the one in the independent measures in the regression model. Instead, a significant relation emerged between GDS-15 depression and adipose tissue C18:3n-3. There were two reasons for including the particular fatty acid as one of the independent variables. One reason is the observed significant correlation between GDS-15 and

this fatty acid. The other reason is that a previous study of this elderly group had indicated a significant inverse relationship between the particular adipose fatty acid and GDS-15 (Mamalakis *et al.*, 2004). However, serum cholesteryl ester fatty acid measures had not been included in the statistical analysis at that time (Mamalakis *et al.*, 2004). The adipose tissue is a biomarker of long term (1–3 years) or habitual dietary fat intake (Dayton *et al.*, 1966; Beynen *et al.*, 1980). On the other hand, serum cholesteryl esters is a biomarker of fatty acid intake of the preceding 1–2 weeks (Katan *et al.*, 1997). Based on our findings, it appears that depression is more strongly related to long-term than to short-term fatty acid intake. A possible explanation may relate to the rate of deposition of dietary PUFA to the nervous system. Animal studies have shown that as a result of dietary replacement regimens, there was a relatively slow rate of replacement of long-chain PUFA to the brains of animals previously depleted (dietary restriction/deprivation studies) in the particular fatty acids (Bourre *et al.*, 1987; Connor *et al.*, 1990; Moriguchi *et al.*, 2001). The speed of recuperation from PUFA depletion was slower in the brain compared to other tissues (Moriguchi *et al.*, 2001). It has been reported that, in animals, total recovery of brain PUFA takes several weeks to complete (Youyou *et al.*, 1986; Bourre *et al.*, 1987; Homayoun *et al.*, 1988; Connor *et al.*, 1990; Moriguchi *et al.*, 2001). It is estimated that there is a 0.3% daily replacement rate for arachidonic acid in the human brain (Rapoport *et al.*, 2001). Based on these observations, it may be reasonable to assume that brain fatty acids reflect long-term rather than short-term fatty acid intake and are thus more strongly related to adipose tissue than to serum cholesteryl ester fatty acids. However, this assumption has not been tested yet. Nevertheless, relative to serum cholesteryl ester fatty acids, adipose ones may be more strongly related to brain fatty acids and depression, thereto. This is the first study that has investigated both serum cholesteryl ester and adipose tissue fatty acids in relation to depression. The implication of our findings is that relative to biomarkers of short-term fatty acid intake, biomarkers of long-term fatty acid intake is a better index of the true relation between fatty acids and depression. Furthermore, biomarkers of long-term fatty acid intake may provide a better estimate than those of short-term one, of the true relation between dietary fatty acids and human brain diseases. More studies are needed to confirm our findings.

Depression is characterized by elevated cytokines such as IL-1, IL-6 and tumor necrosis factor (TNF)-alpha (Maes *et al.*, 1993; Musselman *et al.*, 2001; Hestad *et al.*, 2003). On the other hand, human studies have reported reductions in IL-1, IL-6 and TNF-alpha synthesis as a result of dietary supplementation with C18:3n-3 (Caughey *et al.*, 1996; James *et al.*, 2000; Rallidis *et al.*, 2003). The observed inverse relationship between adipose tissue C18:3n-3 and depression, therefore, may be mediated by decreases in IL-1, IL-6 and TNF-alpha levels. To the best of our knowledge, there are no literature reports that depression is associated with aversion for certain foods (e.g. sea food). Often, depression is associated with diminished appetite, food and caloric intake, and weight loss (Kazes *et al.*, 1994; Antonijevic *et al.*, 1997). However, this is unlikely to be the case in our depressed sub-sample. The reason is that reduced food and energy intake is accompanied by decreases in BMI and serum cholesteryl ester C18:2n-6, and increases in serum cholesteryl ester C16:0 and C20:4n-6 fatty acids, a fact not evidenced in the depressed sub-sample relative to the non-depressed one (Schouten *et al.*, 1981; Rossner *et al.*, 1989; Phinney *et al.*, 1991). Furthermore, the fact that the entire sample consisted of farmers, rules out the possibility that the reduced adipose tissue C18:3n-3 levels in our depressed sub-sample have been reflecting socioeconomic differences from the non-depressed one. Finally, there was no health or nutrition education intervention component in the Seven Countries Study. Therefore, it is considered unlikely that the subjects' participation in the Seven Countries Study may have had an influence on their dietary habits. The

observed inverse relationship between adipose tissue C18:3n-3 and depression indicates that a reduced long-term C18:3n-3 intake is associated with an elevated risk for depression in the elderly.

In conclusion, the positive relationship between serum cholesteryl ester AA/DHA ratio and depression may reflect the opposing effects of AA and DHA on PGE_2 production. Another reason for the observed positive relationship may be the reported opposing effects of AA and DHA on neuron integrity in the hippocampus. It appears that the fatty acids of the adipose tissue are better predictors of depression than those of serum cholesteryl esters. This indicates that depression relates more strongly to long-term than to short-term fatty acid intake. The reason for this may be the reported slow rate of deposition of dietary PUFA to the brain. The inverse relationship between adipose tissue C18:3n-3 and depression may be mediated by reductions in IL-1, IL-6 and TNF-alpha levels. This inverse relationship indicates that a reduced long-term C18:3n-3 intake is associated with an elevated risk for depression in the elderly.

References

1. 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.
2. Ahmad A, Moriguchi T, Salem N (2002). Decrease in neuron size in docosahexaenoic acid-deficient brain. Pediatr Neurol 26, 210–218.
3. Almeida OP, Almeida SA (1999). Short versions of the geriatric depression scale: a study of their validity for the diagnosis of a major depressive episode according to ICD-10 and DSM-IV. Int J Geriatr Psychiatr 14, 858–865.
4. Antonijevic IA, Murck H, Frieboes RM, Horn R, Brabant G, Steiger A (1997). Elevated nocturnal profiles of serum leptin in patients with depression. J Psychiatr Res 32, 403–410.
5. Beynen AC, Katan MB (1985). Why do polyunsaturated fatty acids lower serum cholesterol? Am J Clin Nutr 42, 560–563.
6. 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.
7. Bourre JM, Youyou A, Durand G, Pascal G (1987). Slow recovery of the fatty acid composition of sciatic nerve in rats fed a diet initially low in n-3 fatty acids. Lipids 22, 535–538.
8. Calabrese JR, Skwerer RG, Barna B, Gulledge AD, Valenzuela R, Butkus A *et al*. (1986). Depression, immunocompetence, and prostaglandins of the E series. Psychiatr Res 17, 41–47.
9. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ (1996). The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 63, 116–122.
10. Colin A, Reggers J, Castronovo V, Ansseau M (2003). Lipids, depression and suicide. Encephale 29, 49–58.
11. Connor WE, Neuringer M, Lin DS (1990). 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 31, 237–247.
12. 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.
13. 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.
14. Fountoulakis KN, Tsolaki M, Iacovides A, Yesavage J, O'Hara R, Kazis A *et al*. (1999). The validation of the short form of the Geriatric Depression Scale (GDS) in Greece. Aging Clin Exp Res 11, 367–372.
15. 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.
16. 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.
17. Hibbeln JR (1998). Fish consumption and major depression. Lancet 351, 1213.

18. Himmelseher S, Pfenninger E, Georgieff M (1996). The effect of basic fibroblast growth factor on glutamate-injured neuroarchitecture and arachidonic acid release in adult hippocampal neurons. Brain Res 707, 54–63.
19. Homayoun P, Durand G, Pascal G, Bourre JM (1988). Alteration in fatty acid composition of adult rat brain capillaries and choroid plexus induced by a diet deficient in n-3 fatty acids: slow recovery after substitution with a nondeficient diet. J Neurochem 51, 45–48.
20. 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.
21. James MJ, Gibson RA, Cleland LG (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 71 (Suppl 1), 343–348.
22. 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.
23. Kazes M, Danion JM, Grange D, Pradignac A, Simon C, Burrus-Mehl F *et al*. (1994). Eating behaviour and depression before and after antidepressant treatment: a prospective, naturalistic study. J Affect Disord 30, 193–207.
24. Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Ferretti A, Erickson KL *et al*. (1999). Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. Lipids 34, 317–324.
25. Kim HC, Jhoo WK, Bing G, Shin EJ, Wie MB, Kim WK *et al*. (2000). Phenidone prevents kainate-induced neurotoxicity via antioxidant mechanisms. Brain Res 874, 15–23.
26. Krakauer KA, Williamson PK, Baker DG, Zurier RB (1986). Separation and quantification of prostaglandins E1 and E2 as their panacyl derivatives using reverse phase high pressure liquid chromatography. Prostaglandins 32, 301–310.
27. Lieb J, Karmali R, Horrobin D (1983). Elevated levels of prostaglandin E2 and thromboxane B2 in depression. Prostag Leukot Med 10, 361–367.
28. Linnoila M, Whorton AR, Rubinow DR, Cowdry RW, Ninan PT, Waters RN (1983). CSF prostaglandin levels in depressed and schizophrenic patients. Arch Gen Psychiatry 40, 405–406.
29. Lokesh BR, Kinsella JE (1987). Modulation of prostaglandin synthesis in mouse peritoneal macrophages by enrichment of lipids with either eicosapentaenoic or docosahexaenoic acids *in vitro*. Immunobiology 175, 406–419.
30. Maes M, Bosmans E, Meltzer HY, Scharpe S, Suy E (1993). Interleukin-1 beta: a putative mediator of HPA axis hyperactivity in major depression? Am J Psychiatry 150, 1189–1193.
31. 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. Psychiatry Res 85, 275–291.
32. 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.
33. Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A (2004). 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.
34. Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A (2004a). Depression and adipose polyunsaturated fatty acids in an adolescent group. Prostagl Leukotr Essent Fatty Acids 71, 289–294.
35. Mamalakis G, Tornaritis M, Kafatos A (2002). Depression and adipose essential polyunsaturated fatty acids. Prostagl Leukotr Essent Fatty Acids 67, 311–318.
36. Metcalfe LD, Schmitz AA, Pekka JR (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Ann Chem 18, 514–515.
37. Moriguchi T, Loewke J, Garrison M, Salem Jr N (2001). Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum. J Lipid Res 42, 419–427.
38. Muller N, Riedel M, Schwarz MJ (2004). Psychotropic effects of COX-2 inhibitors – a possible new approach for the treatment of psychiatric disorders. Pharmacopsychiatry 37, 266–269.
39. Musselman DL, Miller AH, Porter MR, Manatunga A, Gao F, Penna S *et al*. (2001). Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. Am J Psychiatr 158, 1252–1257.
40. Myint AM, Kim YK (2003). Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. Med Hypotheses 61, 519–525.
41. Nakane Y, Ohta Y, Uchino J, Takada K, Yan HQ, Wang XD *et al*. (1988). Comparative study of affective disorders in three Asian countries. Acta Psychiatr Scand 78, 698.
42. Nishino S, Ueno R, Ohishi K, Sakai T, Hayaishi O (1989). Salivary prostaglandin concentrations: possible state indicators for major depression. Am J Psychiatr 146, 365–368.
43. O'Hara MW, Kohout FJ, Wallace RB (1985). Depression among the rural elderly. A study of prevalence and correlates. J Nerv Ment Dis 173, 582–589.

44. Ohishi K, Ueno R, Nishino S, Sakai T, Hayaishi O (1988). Increased level of salivary prostaglandins in patients with major depression. Biol Psychiatr 23, 326–334.
45. Osborn DP, Fletcher AE, Smeeth L, Stirling S, Bulpitt CJ, Breeze E *et al*. (2003). Factors associated with depression in a representative sample of 14 217 people aged 75 and over in the United Kingdom: results from the MRC trial of assessment and management of older people in the community. Int J Geriatr Psychiatr 18, 623–630.
46. Paoletti AM, Piccirilli S, Costa N, Rotiroti D, Bagetta G, Nistico G (1998). Systemic administration of *N* omega-nitro-L-arginine methyl ester and indomethacin reduces the elevation of brain PGE2 content and prevents seizures and hippocampal damage evoked by LiCl and tacrine in rat. Exp Neurol 149, 349–355.
47. 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.
48. Phinney SD, Davis PG, Johnson SB, Holman RT (1991). Obesity and weight loss alter serum polyunsaturated lipids in humans. Am J Clin Nutr 53, 831–838.
49. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A (2003). Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. Atherosclerosis 167, 237–242.
50. Rapoport SI, Chang MCJ, Spector AA (2001). Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. J Lipid Res 42, 678–685.
51. Rossner S, Walldius G, Bjorvell H (1989). Fatty acid composition in serum lipids and adipose tissue in severe obesity before and after six weeks of weight loss. Int J Obes Relat Metab Disord 13, 603–612.
52. Sapolsky RM (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biol Psychiatr 48, 713–714.
53. Schouten JA, Van Gent CM, Popp-Snijders C, Van der Veen EA, Van der Voort HA (1981). The influence of low calorie (240 kcal/day) protein–carbohydrate diet on serum lipid levels in obese subjects. Int J Obes Relat Metab Disord 5, 333–339.
54. Shanker G, Hampson RE, Aschner M (2004). Methylmercury stimulates arachidonic acid release and cytosolic phospholipase A2 expression in primary neuronal cultures. Neurotoxicology 25, 399–406.
55. Sheikh JI, Yesavage JA (1986). Geriatric depression scale (GDS): recent evidence and development of a shorter version. Clin Gerontol 5, 165–173.
56. 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.
57. Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamaki H *et al*. (2001). Fish consumption and depressive symptoms in the general population in Finland. Psychiatr Serv 52, 529–531.
58. Vinkers DJ, Stek ML, Gussekloo J, Van Der Mast RC, Westendorp RG (2004). Does depression in old age increase only cardiovascular mortality? The Leiden 85-plus Study. Int J Geriatr Psychiatr 19, 852–857.
59. 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.
60. Youyou A, Durand G, Pascal G, Piciotti M, Dumont O, Bourre JM (1986). Recovery of altered fatty acid composition induced by a diet devoid of n-3 fatty acids in myelin, synaptosomes, mitochondria, and microsomes of developing rat brain. J Neurochem 46, 224–228.