# N-3 polyunsaturated fatty acids in adipose tissue and depression in different age groups from Crete

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Dit onderzoek is uitgevoerd binnen de onderzoeksschool VLAG.

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**Proefschrift**

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
Prof.dr. M.J. Kropff
in het openbaar te verdedigen
op woensdag 13 juni 2007
des namiddags te 13.30 uur in de Aula

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Thesis Wageningen University, Wageningen, The Netherlands

ISBN: 978-90-8504-662-2

# ABSTRACT

In this thesis, the results of cross-sectional studies on the relationship of depression with adipose tissue n-3 polyunsaturated fatty acids (n-3 PUFA) have been described. The aim of this thesis is to investigate whether adipose tissue n-3 fatty acids, an objective index or biomarker of long-term or habitual n-3 PUFA intake relates to depression. The study populations in this thesis consisted of generally healthy adolescent, adult and elderly volunteers from the island of Crete. With the exception of the elderly group that consisted solely of males, all other age-groups consisted of both male and female subjects.

Significant relationships between different n-3 PUFA and depression were not manifested in our adolescent group. In the same adolescent group, inclusion of serum adiponectin along with other covariates in the statistical analysis, yielded a significant inverse relationship ($r=-0.24$, $p<0.05$) between scores on the Beck Depression Inventory (BDI) and adipose tissue eicosapentaenoic acid (EPA).

A significant inverse association between depression and adipose tissue docosahexaenoic acid (DHA) was observed in one of our adult groups. A comparison of mildly depressed adults and non-depressed ones indicated significantly lower (-34.6%, $p<0.05$) adipose tissue DHA levels in the former sub-group. The inverse relationship between depression and adipose tissue DHA was also confirmed in a second adult group. Still, in another adult group, depression was not significantly related to adipose tissue n-3 PUFA.

A significant inverse relationship between depression and adipose tissue alpha-linolenic acid (ALA) was observed in a group of elderly subjects. Depressed males had significantly lowered (-10.5%, $p<0.02$) adipose tissue ALA levels than non-depressed ones.

The results presented in this thesis demonstrated for the fist time in the different age-groups studied, significant inverse relationships between depression and different adipose tissue n-3 PUFA. Adipose tissue EPA was significantly inversely associated with depression in adolescents. Similar relations were found for DHA in adults and for ALA in elderly subjects. However, an all-embracing explanation for the relations observed for the different n-3 PUFA in the different age-groups is not yet available.

## CONTENTS

# Chapter 1

# GENERAL INTRODUCTION

Depression, the most common psychiatric disorder in adults [1,2], is a recurrent, [3] costly [4] and highly debilitating disease.[5] Depression has been reported to be characterized by increased morbidity and mortality.[1,6,7] Despite increasing affluence and advances in pharmacotherapy, it has been reported that the age of onset of major depression has decreased, while its incidence has increased, the last 100 years.[8] In a recent publication, the investigators of the Global Burden of Disease project predicted that by 2030, depression will become one of the leading causes of disability worldwide.[9]

Recently, a large pan-European survey of depression (DEPRES I) was conducted. The particular study involved 78,463 adults from six different European countries (UK, Belgium, France, Germany, The Netherlands and Spain). It was found that 13,359 adults or 17% of the population studied, suffered from depression (major depression, minor depression, or depressive symptoms).[10] Another recent study assessed the prevalence of depressive disorders in Europe, in nine randomly selected samples of adults aged 18 to 64. There were 8.764 adult participants from five European countries (UK, Ireland, Norway, Finland and Spain).[11] Depression prevalence (Beck Depression Inventory scores >= 12 and clinical interview) was assessed in both urban and rural samples. The prevalence of depression in the rural communities ranged from 6.5% to 9.3%, while that in the urban communities ranged from 2.6% to 17.1%. Depression prevalence was 8.6% for the entire population (10.1% for women, 6.6% for men).

There are indications that there is a high prevalence of depression among the Greeks. Specifically, a recent study of a randomly selected sample of healthy Greek adults showed that the prevalence of mild-to-severe depression (Zung Self Rating Depression Scale scores >=50) was 25% for men and 33% for women.[12] In addition, a study of healthy elderly people from five European countries (Italy, Greece, France, Poland, Germany) indicated that among the five countries, Greece had the highest prevalence of depression (Geriatric Depression Scale-15 score >=5) (34.4%), followed by Poland (34%), Italy (28.2%), France (10%) and Germany (8.9%).[13]

There is some evidence that the prevalence of depression in Greece is increasing. A cross-sectional survey carried out by the National Center for Social Research in 1978, involved a randomly selected sample of Greek adults from four geographic areas of mainland Greece. The prevalence of mild to severe depression in that group (>16 score on CES-D scale) was 17% (9.6% for males, 23.3% for females).[14] A second cross-sectional survey was carried out by the Department of Psychiatry of Athens University in 1984. The particular study

followed the same methodology as the 1978 survey. A representative sample of Greek adults was recruited from the same areas as the 1978 study. It was found that the prevalence of depression in adult Greeks was significantly higher (10.6%) in 1984 than in 1978. Specifically, the prevalence of mild to severe depression (>16 score on CES-D scale) had increased from 17% in 1978 to 27.6% in 1984. The proportion of depressed males and females in 1984 were 15.4% and 37.6% respectively. The authors attributed the significant increase in depression prevalence between 1978 and 1984, to the psychosocial stress due to the economic instability and recession and increase in inflation rate and unemployment during the particular six-year period.[15] Finally, it has been reported that there has been a significant 73.8% increase in the sales of psychotropic drugs as opposed to an increase of 36% in the sales of all other drugs in Greece, during the particular six-year period.[16]

## DEPRESSION AND N-3 POLYUNSATURATED FATTY ACIDS

The parent compound of the n-3 family of polyunsaturated fatty acids (PUFA) is alpha-linolenic acid (C18:3 n-3) (ALA). ALA is nutritionally an essential fatty acid, because it can not be synthesized by the body or be inter-converted from other fatty acids but must be supplied from the diet. ALA comes primarily from plant sources. Rich sources of ALA are flaxseed, canola and walnuts, and flaxseed oil, walnut oil and rapeseed oil. Once consumed from the diet, ALA undergoes a series of desaturations / elongations to produce its longer-chain derivative fatty acids eicosapentaenoic acid (C20:5 n-3) (EPA), and docosahexaenoic acid (C22:6 n-3) (DHA). It has been reported that humans have a limited capacity of converting ALA to its longer-chain derivatives EPA and DHA.[17,18] It has been suggested that the safest way to ensure adequate supplies / body stores in the later two fatty acids is through exogenous sources or preformed dietary EPA and DHA. Rich sources of dietary EPA and DHA are fish, particularly fatty fish such as sardines, herring, mackerel, salmon, anchovy and tuna, and fish oil.

A number of studies has indicated significant relationships between depression and n-3 PUFA. Specifically, cross-cultural, cross-sectional, case-control and prospective cohort studies have reported inverse relationships between depression and PUFA of the n-3 family.[19-28] Some of these studies have used dietary intake measures such as dietary history or food frequency questionnaires,[22-24] whereas other studies have used objective indicators (biomarkers) of short-term (few days to few months) n-3 PUFA intake.[25-28] These biomarkers of short-term fatty acid intake were red blood cell membrane phospholipids,

plasma and serum phospholipids and plasma and serum cholesteryl esters. For example, case-control studies have shown lower levels of EPA, DHA and total n-3 fatty acids in the red blood cell membrane phospholipids, plasma or erythrocyte blood lipids, and cholesteryl esters of depressive patients as opposed to healthy control subjects.[25-28] Moreover, a number of clinical controlled studies have shown that therapeutic administration of n-3 PUFA to depressed patients over brief time periods, led to significant reductions in depression severity.[29-33] Although most of these studies provide indications for a link between depression and short-term n-3 PUFA intake, no such association has yet been shown for long-term n-3 PUFA intake. In other words, it is not yet known whether a high long-term or habitual n-3 PUFA intake protects against depression. Until now, no studies have investigated the extent to which depression is related to objective indicators or biomarkers of long-term n-3 PUFA intake.

## BIOCHEMICAL MECHANISMS LINKING N-3 PUFA TO DEPRESSION

Although the precise etiology of depression remains unknown, one of the key features of the particular disorder is deficits in adrenergic and serotonergic neurotransmission. Depression has been reported to be associated with reduced norepinephrine and dopamine as well as serotonin levels.[34,35] On the other hand, there are indications that certain n-3 fatty acids may be associated with increases in serotonin and dopamine. Specifically, positive correlations have been reported between plasma DHA and cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations, in healthy humans.[36] Cerebrospinal fluid 5-HIAA and HVA levels reflect central concentrations of serotonin and dopamine respectively.[36] Aside from deficits in adrenergic and serotonergic channels, depression is characterized by inflammation and elevated pro-inflammatory cytokines such as IL-1, IL-2, IL-6 and TNF-alpha.[37-39] On the other hand, it has been reported that n-3 fatty acids such as ALA, EPA and DHA, inhibit the production of the particular pro-inflammatory cytokines in human subjects.[40-44] Finally, it has been reported that depression is associated with neuronal atrophy and volume loss in the hippocampus.[45,46] On the other hand, there are indications that certain fatty acids of the omega-3 series may confer protection to the hippocampus. Specifically, EPA and DHA have been credited with neuroprotective effects on the animal hippocampus.[47-53]

## RATIONALE OF THIS THESIS

When the research for this thesis started, there was some evidence in support of an inverse relationship between depression and n-3 fatty acids. A number of studies that have used objective indices or biomarkers of short-term fatty acid intake have indicated significant inverse relationships between depression and short-term n-3 PUFA consumption.[25-28] Up until now, however, no studies have investigated whether depression relates also to biomarkers of *long-term* or habitual n-3 PUFA intake. The aim of this thesis is to investigate whether adipose tissue n-3 fatty acids, an objective index or biomarker of long-term or habitual n-3 PUFA intake relates to depression.

## STUDY POPULATIONS

The study populations in this thesis were groups of adolescent, adult and elderly subjects from the island of Crete. All groups consisted of healthy, mostly non-depressed, volunteer participants. With the exception of the elderly group that consisted solely of males, all other age-groups consisted of both male and female participants. The adolescent group was comprised of 90 children (54 girls and 36 boys) aged 13 to 18. The group came from an urban community of Crete. One of the adult groups consisted of 247 lawyers (146 males, 101 females) aged 24 to 69. The second adult group, a sub-sample of the Greek ApoEurope study group, consisted of 130 adults (59 males, 71 females) aged 22 to 58. Subjects came from urban and rural communities of Crete. The sample consisted of employees of the University hospital of Crete and the University of Crete, farmers, and third year medical students at the University of Crete. The third adult group, a representative sample of a rural community of Crete, was comprised of 394 subjects (175 males, 219 females) aged 18 to 60. The particular subjects were involved into small farming business. The elderly group consisted of 150 male survivors of the Cretan cohort of the Seven Countries Study.

## MEASURES OBTAINED

Anthropometric measures such as body weight and height were taken, and body mass index (BMI) was calculated by dividing weight (Kg) by height squared ($m^2$). In addition, data concerning subjects' smoking habits and education were collected.

Long-term or habitual n-3 fatty acid intake was assessed through the use of the adipose tissue as a biological specimen or compartment. Both, adipose tissue and erythrocyte

membranes, serum triacylglycerols, cholesteryl esters and phospholipids, all have been used as objective biological indicators or biomarkers of fatty acid intake. However, serum triacylglycerol, cholesteryl ester and phospholipid and erythrocyte membrane fatty acid composition reflects fatty acid intake of the preceding few hours to several weeks.[54] By contrast, the adipose tissue has a very slow turnover (0.11% daily) and a half-life of approximately 600 days.[55-57] The fatty acid composition of adipose tissue has been reported to reflect dietary intake of the preceding 2 to 3 year period.[55,58] Adipose samples were aspirated from the gluteal adipose tissue (buttock) and n-3 PUFA were determined by gas chromatography. There are indications that abdominal adipose tissue has a faster fatty acid turn over rate than gluteal depots.[57,59] Consequently, gluteal adipose tissue should perhaps be preferred over abdominal adipose tissue for the study of long-term fatty acid consumption.

Adipose tissue is a useful marker of the type of fat consumed.[60,61] However, adipose tissue has a limited value as a dietary intake index for monounsaturated and saturated fatty acids.[62] The reason is that the adipose levels of these nonessential fatty acids are not only the result of dietary intake but also of endogenous synthesis. However, this is not the case with trans fatty acids, the odd-chain saturated fatty acids (e.g. C15:0 and C17:0) and the essential polyunsaturated fatty acids (n-3 and n-6 PUFA), that can not be synthesized endogenously but are derived exclusively from dietary sources.[63,64] It has been reported that the adipose tissue is a particularly useful dietary intake marker for essential fatty acids such as the n-3 PUFA.[65] Adipose tissue n-3 PUFA levels have been reported to closely reflect dietary intake levels.[66-70]

The use of dietary intake measures such as dietary history or food-frequency questionnaires or estimated or weighed food records is flawed with bias due to inaccurate recall and reporting, changes in seasonal eating patterns, inherent limitations in the use of the food consumption tables used to assess intake of individual fatty acids, and over- or under-reporting of foods regarded as more socially acceptable or less socially acceptable respectively. The use of the adipose tissue, on the other hand, is free of such bias. Actually, the adipose tissue has been used to validate dietary questionnaires.[71,72] Owing to its slow turnover [55-57] and insensitivity to acute disease,[73,74] adipose tissue is perhaps an ideal medium for the study of long-term fatty acid intake. The use of the adipose tissue as a biomarker of long-term fatty acid intake may be especially suited for the study of chronic disease or diseases that can have a long course and duration such as depression. However, a

weakness in using adipose tissue measures is that these measures represent qualitative rather than quantitative estimates. In other words, the different individual fatty acids assessed reflect relative or qualitative (% of the total fatty acids in the chromatogram) rather than absolute or quantitative (e.g. mg) fatty acid intake. Nevertheless, the adipose tissue fatty acid content has been used in epidemiological research as a valid marker of the type of fat consumed by different populations [58] and within individuals.[75,76]

Depressive symptomatology was assessed through the administration of well-known, widely used self-rating depression scales such as the Beck Depression Inventory (BDI),[77] the Zung Self Rating Depression Scale (ZSRDS),[78] the Center for Epidemiologic Studies Depression Scale (CES-D) [79] and the 15-item version of the Geriatric Depression Scale (GDS-15).[80] The rationale for selecting the particular instruments were their well established psychometric properties (reliability and validity) as well as their applicability to the particular age-groups studied.[81-86] Furthermore, the CES-D,[87] the ZSRDS [88] and the GDS-15 [89] have been standardized in Greeks.

## OUTLINE OF THIS THESIS

In this thesis, cross-sectional studies on the relations between depression and n-3 PUFA are addressed. Seven studies describe the relationship between degree of depression or depressive symptomatology and n-3 PUFA in the adipose tissue, an objective indicator or biomarker of habitual or long-term intake, in different age-groups. In **CHAPTERS 2** and **3** we report on the association between depression and adipose tissue n-3 PUFA in an adolescent group.

In **CHAPTER 4**, the relationship between degree of depression and adipose omega-3 PUFA is investigated in a group of adults (lawyers). In addition, comparisons are made between mildly depressed subjects and non-depressed ones in adipose tissue n-3 PUFA composition. In **CHAPTER 5**, we investigate for possible associations between depression and adipose n-3 PUFA in an adult sub-sample of the Greek ApoEurope study group. This sample consisted of 130 adults (59 males, 71 females) from urban and rural communities of Crete. In **CHAPTER 6**, the relationship of depression with adipose n-3 fatty acids is investigated in an adult population**,** a representative sample of a rural community of Crete.

In **CHAPTER 7**, the association between degree of depression symptomatology and adipose tissue n-3 PUFA composition is investigated in the surviving elderly males of the

Cretan cohort of the Seven Countries Study. Furthermore, comparisons are made between depressed and non-depressed subjects in adipose tissue n-3 fatty acid composition.

In **CHAPTER 8**, we investigate whether the relationship between depression and n-3 PUFA in adipose tissue in the elderly survivors of the Greek Seven Countries Study group persists, after controling for serum cholesteryl ester fatty acid composition.

Finally, the main results, methodological considerations and the strength of the associations between different n-3 PUFA and depression are discussed in **CHAPTER 9**.

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# Chapter 2

# Depression and adipose n-3 polyunsaturated fatty acids in an adolescent group

Published as: Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A. Depression and adipose polyunsaturated fatty acids in an adolescent group. Prostagl Leukotr Essent Fatty Acids 2004; 71: 289-94.

**ABSTRACT**

The purpose of the present study was to investigate the relation between adipose tissue polyunsaturated fatty acids, an index of long-term or habitual fatty acid dietary intake and depression. The sample consisted of 90 adolescents from the island of Crete. There were 54 girls and 36 boys, aged 13 to 18. The mean age was 15.2 years. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. Depression was assessed through the use of the Beck Depression Inventory (BDI) and the Center for Epidemiologic Studies Depression Scale (CES-D). Unlike other studies, there were no significant relations between adipose tissue n-3 or n-6 polyunsaturated fatty acids and depression. BDI correlated positively with adipose tissue C20:3n-6 / C18:3n-6 ratio, while CES-D correlated positively with adipose tissue (C20:3n-6 + C22:5n-3) / (C18:3n-6 + C20:5n-3) ratio. Depressed subjects (BDI>16, CES-D>16) had significantly elevated adipose tissue C20:3n-6 / C18:3n-6 and (C20:3n-6 + C22:5n-3) / (C18:3n-6 + C20:5n-3) ratios, than non-depressed subjects. The observed positive relation between depression and the particular fatty acid ratios, in the present study, appears to indicate increasing activity of elongases, the enzymes responsible for elongating polyunsaturated fatty acids into their longer-chain derivatives, with increasing depression. This is the first literature report of a possible relation between elongases and depression. The observed relation may stem from a possible over-expression of the HELO1 (ELOVL5) gene, the gene encoding a protein responsible for elongating long-chain polyunsaturated fatty acids, in the adipose tissue of depressed adolescents.

## INTRODUCTION

Depression has been one of the major health problems of the last century. [1-2] It appears that decreases in depression prevalence are associated with increased consumption of fish. [3-4] There are indications, that depletions in docosahexaenoic acid (C22:6n-3) (DHA), one of the long-chain polyunsaturated fatty acids (PUFA) in fish-oil, [5] as well as other long-chain n-3 PUFA may be associated with depression. Significant depletions in red blood cell membrane phospholipid DHA and other n-3 long-chain PUFA, have been reported in depressed patients as opposed to healthy controls. [6-7] Furthermore, dietary intake of n-3 PUFA as well as red blood cell membrane PUFA levels have been reported to correlate negatively with depression severity.[7] As indicated by another study, erythrocyte phospholipid eicosapentaenoic acid (C20:5n-3) (EPA) levels correlated negatively with depression severity, in a group of depressed patients.[8]

Besides polyunsaturated fatty acids of the n-3 series, PUFA of the n-6 family, also have been reported to relate to depression. Specifically, positive correlations have been reported between the ratio of n-6 polyunsaturated arachidonic acid (c20:4 n-6) (AA) to EPA, as well as the ratio of total n-6/n-3 PUFA in erythrocytes, and depression severity.[8] In another study, major depressed patients had significantly elevated phospholipid and cholesteryl ester AA/EPA ratios and cholesteryl ester n-6/n-3 fatty acid ratios, than minor depressed patients or healthy controls. Major depressed patients had significantly decreased serum cholesteryl ester n-3 PUFA and cholesteryl ester and phospholipid EPA, than minor depressed patients or healthy controls.[9] Finally, significantly increased AA/EPA ratios and significantly decreased n-3 PUFA have been reported in phospholipids and serum cholesteryl esters of depressed patients as opposed to healthy controls.[10]

However, not all studies have shown decreased n-3 PUFA in depressed patients as opposed to healthy subjects. Specifically, two studies have shown significant increases rather than decreases in plasma choline phosphoglyceride and erythrocyte EPA and DHA levels in depressed patients as opposed to healthy control subjects.[11-12] Nevertheless, since plasma phospholipids and cholesteryl esters are markers of fatty acid intake of the preceding few weeks,[13-14] the decreased n-3 PUFA in depression reported by most studies, appears to reflect, in part, a corresponding reduced intake in the particular fatty acids.

Some other reason for the reported reductions of n-3 PUFA in depression, may relate to some pathophysiological features of this disease, namely inflammation and lipid peroxidation, and low zinc concentrations.[15-16] It is known that inflammatory response

system activation is associated with lipid peroxidation and reduced levels of n-3 PUFA.[16-17] Similarly, reduced levels of zinc, an inflammation marker, an antioxidant and a co-factor to the formation of desaturated and elongated products of alpha linolenic (C18:3 n-3) acid,[15] are associated with reductions in n-3 PUFA.[15] It is possible, therefore, that the reported reductions in n-3 PUFA in depression, may relate to the particular pathophysiological features of the disease.

It is worth noting that only two studies used adipose tissue fatty acid measures, a biomarker of long-term (1 to 3 year) or habitual dietary fat intake. [18-19] One of these studies indicated that adipose tissue DHA related negatively to depression in an adult group.[20] The other study indicated an inverse relation between adipose tissue alpha-linolenic acid (C18:3n-3) and depression, in an elderly group.[21] However, no study has as yet been conducted on the relation between adipose tissue fatty acids and depression in adolescents.

The aim of the present study was to examine the relation between depression and adipose tissue PUFA of the n-3 and n-6 families in a group of adolescents.

## METHODS

### Study population

The study sample consisted of 90 adolescents from the island of Crete. There were 54 girls and 36 boys, aged 13 to 18. Most of the subjects (81%) were between 13 and 16.5 years of age. The mean age was 15.2 years. 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.

### Depression assessment

Depression level was assessed through the use of a Greek translation of the Beck Depression Inventory (BDI) and the Center for Epidemiologic Studies Depression Scale (CES-D). (BDI), a 21-item scale, has been reported to constitute a valid and reliable depression measure in adolescents. [22-24] CES-D, a 20-item scale, is a valid and reliable measure of depression in adolescents. [25-26] Furthermore, CES-D has been standardized in Greeks. [27]

**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 (m2).

**Adipose tissue measures**

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan.[28] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[28] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids. [28] 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$^{o}$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. [29] Briefly, 20-30 mg of fat sample were saponified with 1.0 ml NaOH in methanol and the FAME were prepared with 14% boron trifluoride in methanol followed by extraction with hexane after washing with saturated NaCl. The hexane (upper layer) containing the FAME was transferred to GC vials and stored at –20$^{o}$ C until analysis. The FAME were separated on a 100 x 0.25 mm Id.SP-2560 fussed 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$^{o}$ C, FID 260$^{o}$ C, split ratio 1:4 to 1:20 (depending on the sample quantity), and oven temperature from 140$^{o}$ C to 245$^{o}$ C with stepped temperature program: within total run time 54 min.

**Statistical methods**

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

## RESULTS

Table 2.1 depicts means and standard deviations of depression, age, anthropometric, and adipose tissue fatty acid measures in the two genders, while Table 2.2 depicts means and standard deviations of the particular variables in depressed v/s non-depressed subjects. Depressed subjects had significantly higher elongation indices. Specifically, depressed adolescents (BDI>16) had higher adipose tissue C20:3n-6/C18:3n-6 ($P<0.05$) and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ($P<0.05$) ratios than non-depressed ones. Also, depressed adolescents (CES-D>16) had higher adipose C22:5n-3/C20:5n-3 ($P<0.05$) and

**Table 2.1** Depression, anthropometric, and adipose tissue fatty acid measures (mean ± standard deviation) in the two genders

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| | Girls | | | Boys | | |
|---|---|---|---|---|---|---|
| | *Mean* | *SD* | *N* | *Mean* | *SD* | *N* |
| Age | 15.5 | 1.6 | 54 | 14.8 | 1.4 | 36 |
| BMI | 22.06 | 4.20 | 54 | 24.21 | 4.59 | 36 |
| BDI | 10.51 | 7.24 | 54 | 7.13 | 5.23 | 36 |
| CES_D | 17.27 | 11.32 | 54 | 11.30 | 7.67 | 36 |
| C18:2n-6 | 13.00 | 1.70 | 54 | 13.30 | 2.31 | 36 |
| C18:3n-6 | 0.07 | 0.01 | 54 | 0.06 | 0.02 | 36 |
| C20:2n-6 | 0.17 | 0.02 | 54 | 0.18 | 0.04 | 36 |
| C20:3n-6 | 0.20 | 0.05 | 54 | 0.21 | 0.05 | 36 |
| C20:4n-6 | 0.35 | 0.07 | 54 | 0.36 | 0.11 | 36 |
| C18:3n-3 | 0.51 | 0.05 | 54 | 0.53 | 0.07 | 36 |
| C20:3n-3 | 0.03 | 0.01 | 54 | 0.03 | 0.01 | 36 |
| C20:5n-3 | 0.02 | 0.01 | 54 | 0.03 | 0.01 | 36 |
| C22:3n-3 | 0.11 | 0.03 | 54 | 0.12 | 0.03 | 36 |
| C22:5n-3 | 0.09 | 0.03 | 54 | 0.10 | 0.03 | 36 |
| C22:6n-3 | 0.09 | 0.03 | 54 | 0.10 | 0.04 | 36 |
| sum n-3 fatty acids | 0.86 | 0.11 | 54 | 0.92 | 0.10 | 36 |
| sum n-6 fatty acids | 14.08 | 1.70 | 54 | 14.36 | 2.35 | 36 |
| n-6/n-3 ratio | 16.65 | 3.11 | 54 | 15.85 | 3.19 | 36 |

(C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) (P<0.05) ratios than their non-depressed counterpart (Table 2.2).

**Table 2.2** Anthropometric, and adipose tissue fatty acid measures (mean ± standard deviation) in depressed (BDI>16, CES-D>16) v/s non-depressed adolescents**.**

| | BDI | | | | | | CES-D | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-depressed | | | Depressed (BDI>16) | | | Non-depressed | | | Depressed (CES-D>16) | | |
| | *Mean* | *SD* | *N* | *Mean* | *SD* | *N* | *Mean* | *SD* | *N* | *Mean* | *SD* | *N* |
| AGE | 15.3 | 1.6 | 71 | 14.7 | 1.2 | 12 | 15.4 | 1.6 | 50 | 15.0 | 1.5 | 34 |
| BMI | 22.43 | 4.05 | 71 | 24.15 | 4.91 | 12 | 22.79 | 3.85 | 50 | 23.35 | 5.27 | 34 |
| C18:2n-6 | 13.10 | 2.00 | 71 | 13.38 | 1.96 | 12 | 13.26 | 2.32 | 50 | 13.00 | 1.48 | 34 |
| C18:3n-6 | 0.07 | 0.01 | 71 | 0.06 | 0.01 | 12 | 0.07 | 0.02 | 50 | 0.06 | 0.01 | 34 |
| C20:2n-6 | 0.18 | 0.03 | 71 | 0.18 | 0.03 | 12 | 0.18 | 0.04 | 50 | 0.17 | 0.03 | 34 |
| C20:3n-6 | 0.20 | 0.05 | 71 | 0.22 | 0.06 | 12 | 0.20 | 0.05 | 50 | 0.22 | 0.06 | 34 |
| C20:4n-6 | 0.35 | 0.09 | 71 | 0.36 | 0.07 | 12 | 0.36 | 0.09 | 50 | 0.36 | 0.08 | 34 |
| C18:3n-3 | 0.52 | 0.06 | 71 | 0.53 | 0.08 | 12 | 0.53 | 0.05 | 50 | 0.51 | 0.07 | 34 |
| C20:3n-3 | 0.03 | 0.01 | 71 | 0.03 | 0.01 | 12 | 0.03 | 0.01 | 50 | 0.03 | 0.01 | 34 |
| C20:5n-3 | 0.03 | 0.01 | 71 | 0.02 | 0.01 | 12 | 0.03 | 0.01 | 50 | 0.02 | 0.01 | 34 |
| C22:3n-3 | 0.11 | 0.03 | 71 | 0.12 | 0.04 | 12 | 0.11 | 0.03 | 50 | 0.12 | 0.04 | 34 |
| C22:5n-3 | 0.10 | 0.03 | 71 | 0.10 | 0.02 | 12 | 0.10 | 0.03 | 50 | 0.10 | 0.03 | 34 |
| C22:6n-3 | 0.09 | 0.03 | 71 | 0.09 | 0.03 | 12 | 0.09 | 0.04 | 50 | 0.09 | 0.03 | 34 |
| sum n-3 fatty acids | 0.88 | 0.11 | 71 | 0.89 | 0.11 | 12 | 0.89 | 0.11 | 50 | 0.88 | 0.11 | 34 |
| sum n-6 fatty acids | 14.17 | 2.02 | 71 | 14.46 | 1.95 | 12 | 14.32 | 2.35 | 50 | 14.10 | 1.49 | 34 |
| n-6/n-3 ratio | 16.33 | 3.13 | 71 | 16.61 | 3.74 | 12 | 16.39 | 3.50 | 50 | 16.28 | 2.86 | 34 |
| C20:3n-6/C18:3n-6 | 3.08 | 0.98 | 71 | 3.73***** | 1.15 | 12 | 3.02 | 1.01 | 50 | 3.46 | 1.04 | 34 |
| C22:5n-3/C20:5n-3 | 4.06 | 1.21 | 71 | 4.34 | 1.34 | 12 | 3.91 | 1.21 | 50 | 4.51▲ | 1.19 | 34 |
| (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) | 3.29 | 0.88 | 71 | 3.86***** | 1.13 | 12 | 3.22 | 0.90 | 50 | 3.69▲ | 0.97 | 34 |

Comparisons are made against the non-depressed category (one-way ANOVA)
*****P<0.05, ▲P<0.05

Table 2.3 depicts Pearson correlations between depression and adipose tissue fatty acids. Depression (BDI and CES-D) scores correlated positively with adipose tissue C20:3n-6/C18:3n-6 (P<0.05) and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) (P<0.05) ratios.

**Table 2.3** Pearson correlations between depression and adipose tissue fatty acids.

| | *BDI* | *CES_D* |
|---|---|---|
| C18:2n-6 | -.007 | .003 |
| C18:3n-6 | -.158 | -.125 |
| C20:2n-6 | .024 | .053 |
| C20:3n-6 | .145 | .204 |
| C20:4n-6 | .003 | .093 |
| C18:3n-3 | -.054 | -.079 |
| C20:3n-3 | -.070 | .002 |
| C20:5n-3 | -.176 | -.184 |
| C22:3n-3 | .061 | .128 |
| C22:5n-3 | -.057 | .023 |
| C22:6n-3 | -.011 | -.008 |
| sum n-6 fatty acids | -.047 | -.012 |
| sum n-3 fatty acids | -.003 | .015 |
| n-6/n-3 ratio | .035 | .006 |
| C20:3n-6/C18:3n-6 | .245* | .230* |
| C22:5n-3/C20:5n-3 | .114 | .214 |
| (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) | .232* | .253* |

* Correlation is significant at the 0.05 level (2-tailed).

The positive relation between depression and elongation indices was confirmed also by stepwise multiple linear regression analysis. Specifically, 22% of the variability in BDI depression was significantly accounted for by age, sex and adipose tissue C20:3n-6/C18:3n-6 ratio ($F=8.8$, $p<0.0005$). Beta coefficients show that BDI depression is related positively to adipose tissue C20:3n-6/C18:3n-6 ratio and negatively to age and sex. Sex is a dummy variable (females=0, males=1). The major predictor of BDI depression is sex ($B=-0.35$, $t=-3.5$, $P<0.001$), followed by age ($B=-0.34$, $t=-3.4$, $P<0.001$) and adipose C20:3n-6/C18:3n-6 ratio ($B=0.28$, $t=2.8$, $P<0.006$). Similarly, 13% of the variability in CES-D depression was significantly accounted for by sex and adipose tissue (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratio ($F=7.2$, $p<0.001$). Beta coefficients show that CES-D depression is related positively to adipose tissue (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratio and negatively to sex. The major predictor of CES-D depression is sex ($B=-0.3$, $t=-2.9$, $P<0.005$), followed by adipose tissue (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratio ($B=0.27$, $t=2.6$, $P<0.01$).

## DISCUSSION

The results of the present study failed to confirm the negative relation between depression and adipose n-3 polyunsaturated fatty acids reported in adults and the elderly.[20,21] It appears that in this adolescent group, adipose tissue PUFA, an index of relatively long-term PUFA intake,[18-19] is not associated with depression. The reasons for this are not clear and deserve further examination. Previous studies with adult and elderly subjects have indicated inverse relations between depression and adipose tissue docosahexaenoic (C22:6n-3) and alpha-linolenic (C18:3n-3) acids respectively.[20-21] One reason for the failure to observe significant relations between the particular fatty acids and depression in the present study, may be the pronounced differences in the levels of these fatty acids between the adolescent and the previous two groups. Compared to the elderly group, the adolescent group had approximately 44% higher mean C18:3n-3 levels.[21] Compared to the adult group, the adolescent group had approximately 64% lower mean C22:6n-3 levels.[20] It is possible that the inverse relation of depression with the particular fatty acids in the adult and elderly groups may have derived or have been contingent upon the specific C18:3n-3 and C22:6n-3 level ranges observed in these groups. The pronounced discrepancies of the adolescent group from the rest two groups in mean C18:3n-3 and C22:6n-3 levels, therefore, may underlie the failure to obtain a significant relation between these two fatty acids and depression in the former group. Clearly, more studies are needed on the relation between adipose polyunsaturated fatty acids and depression in adolescents.

This study indicated that the degree of depression in adolescents correlated positively with adipose tissue C20:3n-6/C18:3n-6 and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratios (Table 2.3). Furthermore, similar results were obtained when the group was subdivided into depressed v/s non-depressed sub-samples. Specifically, depressed adolescents (BDI>16)[30] had higher adipose tissue C20:3n-6/C18:3n-6 and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratios than non-depressed ones. Also, depressed adolescents (CES-D>16)[26] had higher adipose C22:5n-3/C20:5n-3 and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratios than their non-depressed counterpart (Table 2.2). The particular ratios of polyunsaturated fatty acids probably reflect the activity of elongases, the enzymes responsible for elongating polyunsaturated fatty acids into their longer-chain derivatives.[31] C20:3n-6 and C22:5n-3 are products of chain elongation of C18:3n-6 and C20:5n-3 respectively.[31] The results of this study, therefore, appear to indicate that depression in adolescents is associated with increased chain elongation of adipose tissue C18:3n-6 and

C20:5n-3, into C20:3n-6 and C22:5n-3 respectively. This is the first literature report of a possible relation between elongases and depression.

Recently, HELO1 (alternatively ELOVL5), a gene encoding a protein involved in the elongation of PUFA, was identified in humans.[32-33] The particular gene is located on the short arm of chromosome 6p21.1-p12.1.[32] The highest levels of HELO1 mRNA are in testis and adrenal gland, while substantial amounts of HELO1 mRNA are found also in prostate, lung and brain tissue.[32] Chromosome 6, the largest chromosome sequenced, constitutes approximately 6% of the human genome.[34] Chromosome 6 harbors genes directly implicated, or suspected to be implicated in depression such as the heat shock protein 70 (HSP70-1) gene,[35] the prolyl oligopeptidase (PREP) gene,[36] the serotonin 1B (HTR1B)[37] and 1E (HTR1E)[38] receptor genes, and genes in the HLA region of the major histocompatibility complex.[39] It may be noteworthy that HELO1 is on the same domain (6p21.1-p12.1) of the cytogenetic band, albeit at an approximate distance of 11Mb, as transcriptional regulating protein-132 (TReP-132), a protein implicated in steroid synthesis.[40] Depression has been reported to be characterized by elevated corticosteroidal activity and HPA-axis activation.[41,42] Both HELO1 and TreP-132 are highly expressed in adrenals and testis.[32,40] Cloning of the mouse orthologue of human TReP-132, indicated that expression of the gene was highest in thymus, testis and brain structures such as the hypothalamus, basal ganglia, hippocampus, and piriform and cerebral cortex. The authors concluded that although steroidogenesis pathways have not as yet been firmly established in the brain, expression of TReP-132 in the brain is an anatomical evidence that this gene may be implicated in the de novo steroid synthesis within brain regions involved in behavior and psychiatric disorders.[43] Nevertheless, although proximal genes that share related functions have been identified on the human genome, physical proximity per se does not necessitate functional association among genes.[44]

Should the results of this study be replicated by other adolescent studies, this might instigate a need for investigating the possibility of an over-expression of the HELO1 / ELOVL5 gene in the adipose tissue of depressed adolescents.

In conclusion, the results of the present study indicated that in this adolescent group there was no association between adipose polyunsaturated fatty acids and depression. Instead, there was a significant positive relation between depression and adipose tissue C20:3n-6/C18:3n-6, C22:5n-3/C20:5n-3, and (C20:3n-6+C22:5n-3) / (C18:3n-6+C20:5n-3) ratios. The observed positive relation between depression and the particular fatty acid ratios, appears to indicate increasing activity of elongases, the enzymes responsible for

elongating polyunsaturated fatty acids into their longer-chain derivatives, with increasing depression. The observed relation may stem from a possible over-expression of the HELO1 (ELOVL5) gene, in the adipose tissue of depressed adolescents.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Christos Hatzis, Irene Markatzi and Sofia Flouri.

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# Chapter 3

# Depression and adiponectin and adipose n-3 polyunsaturated fatty acids in adolescents

Published as: Mamalakis G, Kiriakakis M, Tsibinos G, Hatzis C, Flouri S, Mantzoros C, Kafatos A. Depression and serum adiponectin and adipose omega-3 and omega-6 fatty acids in adolescents. Pharmacol Biochem Behav 2006; 85: 474-79.

**ABSTRACT**

The purpose of the present study was to investigate for a possible relationship between depression and serum adiponectin and adipose tissue omega-3 and omega-6 PUFA. The sample consisted of 90 healthy adolescent volunteers from the island of Crete. There were 54 girls and 36 boys, aged 13 to 18. The mean age was 15.2 years. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. Depression was assessed through the use of the Beck Depression Inventory (BDI) and the Center for Epidemiologic Studies Depression Scale (CES-D). Fatty acids were determined by gas chromatography in adipose tissue. CES-D correlated with dihomo-gamma linolenic acid (DGLA). Multiple linear regression analyses showed that BDI was negatively associated with eicosapentaenoic acid (EPA), while CES-D was positively associated with DGLA in adipose tissue. Serum adiponectin was not significantly associated with depression. The negative relationship between adipose EPA and depression in adolescents, is in line with findings of previous studies involving adult and elderly subjects, demonstrating negative relations between depression and adipose omega-3 PUFA. This is the first literature report of a relationship between depression and an individual omega-3 fatty acid in adolescents. The inverse relationship between adipose EPA and depression indicates that a low long-term dietary intake of EPA is associated with an increased risk for depression in adolescents.

## INTRODUCTION

Depression is characterized by an immune-inflammatory response or immune-inflammation markers such as increased number of activated T-cells and peripheral blood cells (i.e. B lymphocytes, CD T-cells, leukocytes, neutrophils and monocytes) and increased pro-inflammatory cytokines such as interleukin-1 (IL-1),[1-5] interleukin-2 (IL-2),[3] interleukin-6 (IL-6),[2,6-9] interferon-γ (INF-γ),[10] tumor necrosis factor-alpha (TNF-a),[11] and increased number of soluble IL-2 (sIL-2R) [1,7,12] and IL-6 (sIL-6R) receptors.[7,13]

Aside from pro-inflammatory cytokines, depression has been reported to relate also to anti-inflammatory cytokines. A study indicated that depressed chronic heart failure patients had lower circulating IL-10 levels, an anti-inflammatory cytokine, than non depressed patients.[14] It has been suggested that the therapeutic efficacy of antidepressants is, among other things, due to anti-inflammatory actions or shifting the pro- / anti-inflammatory cytokine balance towards decreased expression of pro-inflammatory cytokines and increased expression of anti-inflammatory ones.[15-17]

Adiponectin (also known as AdipoQ, Acrp 30, GBP28 or apM1), a novel adipocytokine, is secreted exclusively from adipose tissue.[18-21] The levels of this cytokine in human plasma by far exceed those of any other hormone.[18] Adiponectin, anti-inflammatory cytokine, is inversely related to pro-inflammatory cytokines such as IL-6 and TNF-a, that are reportedly elevated in depression.[8,11,21-24] Also, there are indications that adiponectin may increase the levels of IL-10, an anti-inflammatory cytokine that has been reported to be decreased in depression.[14,25] No studies have as yet examined whether adiponectin relates to depression.

On the other hand, lower proportions of omega-3 PUFA have been reported in the plasma, red blood cell membranes, serum phospholipids and cholesteryl esters and adipose tissue of depressed patients as opposed to healthy controls.[26-32] In addition, there are indications that adiponectin may relate to fatty acids,[33,34] including polyunsaturated fatty acids (PUFA) of the omega-3 family.[34] A previous study did not find a significant relationship between depression and adipose tissue omega-3 PUFA in adolescents.[35] However, adiponectin had not been included as a covariate in the statistical analysis.

The aim of the present study is to examine whether serum adiponectin and adipose PUFA relate to depression in adolescents.

## METHODS

### Study population

Study subjects were 90 healthy adolescent volunteers from the island of Crete. They were enrolled in a secondary school at a low income area of west-side Iraklion. There were 54 girls and 36 boys, aged 13 to 18. The average age was 15.2 years. Subjects and their parents were informed about the nature and the purpose of this study, and a consent form was signed by parents. Children were free to refuse to participate even when there was a signed consent by the parent/legal guardian. 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 polyunsaturated fatty acids in the particular adolescent group (Mamalakis et al, 2004a). The difference between this study and the previous one is that serum adiponectin measures had not been included in the statistical analysis in the previous study.

### 2.2. Depression assessment

Depression level was assessed through the use of the Beck Depression Inventory (BDI) [36]and the Center for Epidemiologic Studies Depression Scale (CES-D).[37] BDI and CES-D have been reported to be valid and reliable depression assessment tools in adolescents.[38,39] Furthermore, CES-D has been standardized in Greeks.[40]

### 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 (m2).

### 2.4. Adiponectin measures

All coded samples were centrifuged and frozen (-70° C) sera were sent in dry ice initially to the coordinating center where they were stored (-70° C) prior to being shipped in one batch to the Beth Israel Deaconess Medical Center, in Boston USA. Blinded adiponectin

measurements were performed by RIA with a sensitivity of 2ng/ml, and intra-assay coefficient of variation of <10% as previously described.[41]

### 2.5. Adipose tissue measures

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan.[42] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[42] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids.[42] 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. Fatty acids analysis was carried out as previously described.[35] The different adipose tissue n-3 and n-6 fatty acids assessed were C18:2n-6, C18:3n-6, C20:2n-6, dihomo-gamma linolenic acid C20:3n-6 (DGLA), C20:4n-6, C18:3n-3, C20:3n-3, eicosapentaenoic acid C20:5n-3 (EPA), C22:5n-3, and docosahexaenoic acid C22:6n-3 (DHA).

### Statistical methods

Data were analyzed through the use of the SPSS statistical package. The statistical methods used were Spearman correlations, partial correlations and linear multiple stepwise Regression analysis. Because BDI and CES-D were not normally distributed, logarithmic (Natural log) transformation of the particular measures was applied.

## RESULTS

Table 3.1 depicts means and standard deviations of depression, adiponectin and adipose tissue PUFA in the two genders and the entire group, while Table 3.2 depicts Spearman correlations between CES-D and BDI and serum adiponectin and adipose PUFA. CES-D correlated with C20:3n-6 (DGLA) ($r=0.24$, $p<0.05$). Serum adiponectin correlated with BMI ($r=-0.27$, $p<0.004$), sex ($r=-0.31$, $p<0.001$), C20:2n-6 ($r=-0.28$, $p<0.02$), DGLA ($r=-0.33$, $p<0.003$), C20:4n-6 ($r=-0.29$, $p<0.009$) and C22:5n-3 ($r=-0.25$, $p<0.03$).

**Table 3.1** Depression, serum adiponectin and adipose tissue fatty acids (mean ± standard deviation) in the study subjects.

| | *Girls* | | | *Boys* | | | *Total* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | (±SD) | N | Mean | (±SD) | N | Mean | (±SD) | N |
| BDI | 10.51 | (±7.24) | 54 | 7.13 | (±5.23) | 36 | 9.1 | (±6.7) | 90 |
| CES-D | 17.27 | (±11.32) | 54 | 11.30 | (±7.67) | 36 | 14.9 | (±10.4) | 90 |
| Adiponectin | 11.9 | (±2.6) | 54 | 10.3 | (±2.5) | 36 | 11.4 | (±2.7) | 90 |
| C18:2n-6 | 13.0 | (±1.70) | 54 | 13.30 | (±2.31) | 36 | 13.1 | (±1.9) | 90 |
| C18:3n-6 | 0.07 | (±0.01) | 54 | 0.06 | (±0.02) | 36 | 0.07 | (±0.01) | 90 |
| C20:2n-6 | 0.17 | (±0.02) | 54 | 0.18 | (±0.04) | 36 | 0.18 | (±0.03) | 90 |
| DGLA† | 0.20 | (±0.05) | 54 | 0.21 | (±0.05) | 36 | 0.20 | (±0.05) | 90 |
| C20:4n-6 | 0.35 | (±0.07) | 54 | 0.36 | (±0.11) | 36 | 0.35 | (±0.09) | 90 |
| C18:3n-3 | 0.51 | (±0.05) | 54 | 0.53 | (±0.07) | 36 | 0.52 | (±0.06) | 90 |
| C20:3n-3 | 0.03 | (±0.008) | 54 | 0.03 | (±0.009) | 36 | 0.03 | (±0.008) | 90 |
| EPA† | 0.02 | (±0.006) | 54 | 0.03 | (±0.007) | 36 | 0.02 | (±0.007) | 90 |
| C22:5n-3 | 0.09 | (±0.03) | 54 | 0.10 | (±0.03) | 36 | 0.1 | (±0.03) | 90 |
| DHA† | 0.09 | (±0.03) | 54 | 0.10 | (±0.04) | 36 | 0.09 | (±0.03) | 90 |

† Dihomo-gamma linolenic acid C20:3n-6 (DGLA)
† Eicosapentaenoic acid C20:5n-3 (EPA)
† Docosahexaenoic acid C22:6n-3 (DHA)

**Table 3.2** Spearman correlations between depression and serum adiponectin and adipose tissue PUFA.

| | *BDI* | *CES-D* |
|---|---|---|
| Serum adiponectin | 0.04 | 0.06 |
| C18:2n-6 | -0.03 | 0.009 |
| C18:3n-6 | -0.07 | -0.09 |
| C20:2n-6 | -0.02 | 0.04 |
| DGLA† | 0.10 | 0.24* |
| C20:4n-6 | 0.003 | 0.15 |
| C18:3n-3 | -0.09 | -0.04 |
| C20:3n-3 | -0.11 | -0.02 |
| EPA† | -0.21 | -0.18 |
| C22:5n-3 | -0.08 | 0.12 |
| DHA† | -0.03 | -0.08 |

* Correlation is significant at the 0.05 level (2-tailed).
† Dihomo-gamma linolenic acid C20:3n-6 (DGLA)
† Eicosapentaenoic acid C20:5n-3 (EPA)
† Docosahexaenoic acid C22:6n-3 (DHA)

Multiple linear regression analysis indicated that 8% of the variability in the log transformed BDI scores was accounted for by age and adipose tissue C20:5n-3 (EPA) ($F=4.2$, $p<0.02$). Beta coefficients show that the log transformed BDI depression scores are related negatively to age ($B=-0.23$, $t=-2.09$, $P<0.04$) and adipose tissue EPA ($B=-0.23$, $t=-2.09$, $P<0.05$). Age and adipose EPA bear equal weights in predicting the dependent measure (Table 3.3).

**Table 3.3** Multiple linear regression analysis. Log transformed Beck depression (BDI) in relation to adipose tissue eicosapentaenoic acid C20:5n-3 (EPA), controlling for age, body mass index (BMI), serum adiponectin, adipose tissue C18:2n-6, C18:3n-6, C20:2n-6, dihomo-gamma linolenic acid C20:3n-6 (DGLA), C20:4n-6, C18:3n-3, C20:3n-3, C22:5n-3 and docosahexaenoic acid C22:6n-3 (DHA) as continous variables, and gender as a dummy variable (females=0, males=1).

| Dependent variable | Predictor | Beta (Standardized coefficients) | t | P |
|---|---|---|---|---|
| *Log transformed (BDI) Depression* | EPA | -0.23 | -2.09 | <0.05 |
| | Age | -0.23 | -2.09 | <0.04 |

Multiple linear regression analysis indicated that 8% of the variability in the log transformed CES-D depression scale was accounted for by sex and adipose tissue DGLA ($F=4.1$, $p<0.02$). Beta coefficients show that the log transformed CES-D scores are related negatively to sex and positively to adipose tissue DGLA. The major predictor of the log transformed CES-D scale is sex ($B=-0.25$, $t=-2.2$, $P<0.04$) followed by DGLA ($B=0.24$, $t=2.1$, $P<0.04$) (Table 3.4).

Partial correlations confirmed the results of linear regressions. Specifically, BDI correlated with EPA ($r=-0.23$, $p<0.05$) and CES-D correlated with DGLA ($r=0.26$, $p<0.03$), after controlling for serum adiponectin.

**Table 3.4** Multiple linear regression analysis. Log transformed Centers for Epidemiologic Studies Depression Scale (CES-D) in relation to adipose tissue dihomo-gamma linolenic acid C20:3n-6 (DGLA), controlling for age, body mass index (BMI), serum adiponectin, adipose tissue C18:2n-6, C18:3n-6, C20:2n-6, C20:4n-6, C18:3n-3, C20:3n-3, eicosapentaenoic acid C20:5n-3 (EPA), C22:5n-3 and docosahexaenoic acid C22:6n-3 (DHA) as continous variables, and gender as a dummy variable (females=0, males=1).

| Dependent variable | Predictor | Beta (Standardized coefficients) | t | P |
|---|---|---|---|---|
| *Log transformed (CES-D) Depression* | Sex | -0.25 | -2.2 | <0.04 |
| | DGLA | 0.24 | 2.1 | <0.04 |

## DISCUSSION

The results of the present study indicate that serum adiponectin does not relate to depression in adolescents. Our results do not parallel those of another study that reported a significant relation between depression and IL-10, another anti-inflammatory cytokine.[14] One reason for the failure to observe a significant relationship between serum adiponectin and depression may relate to the protein encoded by the adiponectin gene. The particular protein shares significant similarities to collagen X and collagen VIII, and complement protein C1q.[43] With the exception of C1q, that appears to be implicated in alzheimer's disease,[44,45] collagens X and VIII have not been reported to be directly implicated in any CNS or brain-related pathologies or psychiatric disorders. Moreover, none of the two adiponectin receptors (i.e. adipor1, adipor2) is highly expressed in the brain. Northern blot analysis of adipor1 and adipor2 mRNA in human tissues has shown that adipor1 is highly expressed in the skeletal muscle but moderately expressed in the brain, whereas adipor2 is highly expressed in the skeletal muscle, liver and placenta, and weakly in brain.[46] Finally, it is possible that the failure to observe a significant relationship between serum adiponectin and depression may relate to the particular age-group studied. More studies on serum adiponectin and depression in adolescents as well as other age-groups are, therefore, needed. This is particularly important in light of the fact that adiponectin is associated with cytokines that are implicated in depression. For example, adiponectin is inversely related to pro-inflammatory cytokines such as IL-6 and TNF-a, that are elevated in depression.[8,11,21-24] In addition, adiponectin may increase the levels of IL-10, an anti-inflammatory cytokine that has been reported to be reduced in depression.[14,25]

Although serum adiponectin did not relate to depression, adipose tissue EPA and DGLA did (Tables 3.3 and 3.4). A previous study involving the particular adolescent group had not yielded a significant relation between these two adipose tissue fatty acids and depression.[35] However, serum adiponectin had not been included as a predictor variable in the particular study. Most probably, serum adiponectin must have served as a confounder variable in the present study, in that its presence as a covariate in the regression model has helped unveil an existing relationship between depression and the particular two adipose fatty acids.[47] Specifically, it has been reported that adiponectin correlates negatively with insulin resistance.[48,49] Given that insulin resistance has not been measured in the present study, it is possible that serum adiponectin, may have served as an indicator of insulin resistance in our adolescents. On the other hand, insulin resistance or impaired insulin sensitivity has been reported to correlate positively to depression.[50,51] In addition, insulin

resistance has been reported to relate inversely to EPA [52,53]and positively to DGLA.[54-56] It is possible therefore, that serum adiponectin may have unmasked a relationship between depression and EPA and DGLA, via its correlating with insulin resistance, a known risk factor of depression and a correlate of EPA and DGLA. However, in the absence of insulin sensitivity measures in the present study, this hypothesis can not be tested.

Given that adipose tissue fatty acid composition is a biomarker of long-term (1 to 3 year) or habitual dietary fat intake,[57,58] the observed inverse relationship between adipose tissue EPA and BDI depression, in the present study, indicates that lower long-term dietary EPA intakes are related to a higher depression risk in adolescents. This is the first literature report of a relationship between depression and an individual omega-3 fatty acid in adolescents. This finding parallels results of previous studies involving different age groups. Negative relationships were reported between depression and adipose tissue docosahexaenoic acid (DHA) levels in an adult sample,[31] and between depression and adipose tissue alpha-linolenic acid (ALA) in an elderly group.[32] The results of the present study and those of previous ones indicate that lower long-term omega-3 fatty acid intake may be related to a higher depression risk in adolescents, the adults and the elderly.

In addition, the inverse relationship between adipose tissue EPA and depression, in the present study, agrees with results of other studies that have shown inverse relationships between consumption of fish and depression.[59-61] Furthermore, the inverse relationship between EPA and depression, is in line with findings of other studies that observed decreased levels of omega-3 fatty acids in plasma, red blood cell membranes and serum cholesteryl esters and phospholipids of depressed patients relative to healthy controls.[26-30] Finally, this finding is in congruence with findings of controlled clinical studies that have shown beneficial effects of omega-3 PUFA administration on depression.[62-64]

It has been reported that n-3 PUFA can suppress some of the pathophysiological features of depression, namely inflammation and immune reactivity markers. Specifically, in vitro studies have shown that EPA and DHA suppress IL-6 production by human endothelial cells,[65,66] and the production of IL-1, IL-2, IL-6, TNF-α and INF-γ by human lymphocytes.[67] As shown by human studies, dietary supplementation with EPA and DHA results in suppressing IL-1, IL-2, IL-6 and TNF-α production by monocytes.[68-71] Given the reported positive relation of depression with cytokines such as IL-1, IL-2, IL-6 and TNF-α,[1-9,11] the observed inverse relationship between adipose tissue EPA and depression, in the present study, may be due to an inhibiting effect of EPA on the production of the particular cytokines.

The observed positive relation between adipose DGLA and CES-D depression, in the present study, is in line with studies that have indicated positive relations between depression and PUFA of the omega-6 family.[26,27,30]

Due to the cross-sectional nature of the present study, no conclusions can be drawn concerning a possible cause-effect relationship between EPA and depression. Whether the inverse relationship between adipose EPA levels and depression in the present study reflects a protective effect of long-term EPA intake on depression or is only an epiphenomenon of depression is not known. However, indications for a possible causal link between omega-3 fatty acids, including EPA, and depression have been provided by double-blind, placebo-controlled clinical trails of omega-3 fatty acids administration in major depression and bipolar disorder,[62-64,72] indicating that these fatty acids may affect, directly or indirectly, on the biochemical substrate of depression.

In conclusion, the results of the present study indicated an absence of a significant association between serum adiponectin and depression. However, after controlling for adiponectin, significant relationships emerged between adipose tissue DGLA and EPA and depression. This is the first literature report of a relationship between depression and an individual omega-3 fatty acid in adolescents. The observed inverse relationship between adipose EPA and depression in adolescents parallels findings of studies involving different age-groups and may be mediated by cytokine release. Thus, a low long-term dietary intake of EPA is associated with an increased risk for depression in adolescents.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Irene Markatzi and Manolis Linardakis.

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# Chapter 4

# Depression and adipose n-3 polyunsaturated fatty acids in an adult group

Published as: Mamalakis G, Tornaritis M, and Kafatos A. Depression and adipose essential polyunsaturated fatty acids. Prostagl Leukotr Essent Fatty Acids 2002; 67: 311-18.

**ABSTRACT**

The objective of the present study was to investigate the relation between adipose tissue polyunsaturated fatty acids, an index of long-term or habitual fatty acid dietary intake and depression. The sample consisted of 247 healthy adults (146 males, 101 females) from the island of Crete. The number of subjects with complete data on all variables studied was 139. Subjects were examined at the Preventive Medicine and Nutrition Clinic of the University of Crete. Depression was assessed through the use of the Zung Self-rating Depression Scale (ZSDS). Mildly depressed subjects had significantly reduced (-34.6%) adipose tissue docosahexaenoic acid (DHA) levels than non-depressed subjects. Multiple linear regression analysis indicated that depression related negatively to adipose tissue DHA levels. In line with findings of other studies, the observed negative relation between adipose tissue DHA and depression, in the present study, appears to indicate increasing long-term dietary DHA intakes with decreasing depression. This is the first literature report of a relation between adipose tissue DHA and depression. Depression has been reported to be associated with increased cytokine production, such as, IL-1, IL-2, IL-6, INF-γ and INF-α. On the other hand, fish oil and omega-3 fatty acids have been reported to inhibit cytokine synthesis. The observed negative relation between adipose DHA and depression, therefore, may stem from the inhibiting effect of DHA on cytokine synthesis.

## INTRODUCTION

Depression constitutes the most common psychiatric disorder in adults and a major health problem in the elderly. [1-2] It has been reported that the incidence of depression has increased, while the age of onset of depression has decreased in the 20th century. [3-5] Depression is associated with increases in all-cause mortality, particularly in men.[1]

It appears that increased consumption of fish is associated with decreases in depression prevalence. [6-7] The main dietary sources of docosahexaenoic acid (c22:6 n-3) (DHA), a long-chain polyunsaturated fatty acid of the n-3 family, are fish and mother's milk. [8] There are indications, that depletions in DHA and other long-chain n-3 polyunsaturated fatty acids may be associated with depression. Compared to healthy controls, depressed patients had significant depletions of red blood cell membrane phospholipid n-3 long-chain polyunsaturates, particularly DHA.[9] In another study, significant depletions of red blood cell membrane n-3 polyunsaturated fatty acids were observed in depressed patients as opposed to healthy controls.[10] Furthermore, red blood cell membrane levels as well as dietary intake of n-3 polyunsaturates correlated negatively with depression severity.[10] Another study reported a significant negative correlation between erythrocyte phospholipid eicosapentaenoic acid (c20:5 n-3) (EPA) levels and depression severity, in a depressed group.[11] However, not only n-3 polyunsaturates, but also polyunsaturated fatty acids of the n-6 family were implicated in depression. Specifically, the ratio of n-6 polyunsaturated arachidonic acid (c20:4 n-6) (AA) to EPA as well as the ratio of total n-6/n-3 polyunsaturates in erythrocytes correlated positively to depression severity.[11] In another study, major depressed patients had significantly elevated n-6/n-3 fatty acid ratios in cholesteryl esters and significantly elevated AA/EPA ratios in both cholesteryl esters and phospholipids than minor depressed patients or healthy controls. Major depressed patients had significantly decreased n-3 polyunsaturates in serum cholesteryl esters and significantly decreased EPA in cholesteryl esters and phospholipids than minor depressed patients or healthy controls.[12] Finally, another study reported significantly increased AA/EPA ratios and significantly decreased n-3 polyunsaturates in serum cholesteryl esters and phospholipids of major depressed patients as opposed to healthy controls.[13] However, not all studies have shown decreases in n-3 polyunsaturated fatty acids in depressed patients as opposed to control subjects. Specifically, two studies have shown significant increases rather than decreases of erythrocyte and plasma choline phosphoglyceride EPA and DHA levels in depressed patients as opposed to healthy controls.[14-15] Nevertheless, given that plasma phospholipids and cholesteryl esters are markers of fatty acid intake of

the past few weeks,[16-17] the decreased n-3 polyunsaturated fatty acids in depression reported by the bulk of the studies, appears to reflect, in part, a corresponding reduced consumption in the particular fatty acids. It is worth noting that none of these studies implemented adipose tissue fatty acid measures, a biomarker of long-term (1 to 3 year) or habitual dietary fat intake. [18-19]

The aim of the present study was to examine the relation between depression and adipose tissue polyunsaturated fatty acids of the n-3 and n-6 families in adults.

## METHODS

### Study population

In an attempt to evaluate the health and nutrition status of the lawyers of Iraklion county, Crete, Greece, it was agreed that all members of the lawyers association of the particular county would participate in a preventive medicine and nutrition program. The study sample consisted of 247 lawyers (146 males, 101 females), while the number of subjects with complete data on all variables was 139. The mean age was 39 years, while most of the subjects were 35 years of age. 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 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. Data concerning dietary habits were collected using the 24-hour dietary recall method.

### Depression assessment

Depression level 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 depression measure. [20-23]

### Anthropometric Measures

Body weight was assayed by a digital scale (Seca) with an accuracy of $\pm$ 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 (m2).

**Adipose tissue measures**

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan.[24] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[24] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids. [24] 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$^{o}$ C. Prior to analysis samples were thawed and the fat was transferred to 10 ml screw-capped tubes with the aim 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. [25] Briefly, 20-30 mg of fat sample were saponified with 0.5 ml NaOH in methanol and the FAME were prepared with 14% boron trifluoride in methanol following extraction with hexane after washing with saturated NaOH. 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 50 x 0.22 mm Id.BPX 70 fusse silica capillary column, coated with a 0.25 μm of cyanopropyl silicone provided by SGE (Melbourne, Australia), using a Hewlett Packkard (HP, Avondale, PA, USA) HP 6890 gas chromatograph equipped with an MSD-5972 mass ionization detector. The HP MS 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) and by reference to mass spectra library. The analytical conditions employed were as follows: volume injected 1 ml, carrier gas helium (1.0 ml/min), injector temperature 230o C, MSD 280o C, split ratio 1:20 to 1:50 (depending on the sample quantity), and oven temperature from 120o C to 245o C with stepped temperature program: within total run time 40 min.

**Statistical methods**

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

## RESULTS

Table 4.1 depicts means and standard deviations of depression, anthropometric, dietary and adipose tissue fatty acid measures in the two genders, while Table 4.2 depicts means and

**Table 4.1** Depression, anthropometric, dietary and adipose tissue fatty acid measures (mean ± standard deviation) in the two different genders.

| | Women | | | Men | | | |
|---|---|---|---|---|---|---|---|
| | N | Mean | SD | N | Mean | SD | Sig |
| Age | 101 | 34.4 | 7.2 | 146 | 42.1 | 10.5 | P<0.0005 |
| BMI | 107 | 23.4 | 3.4 | 145 | 29.2 | 24.6 | P<0.016 |
| Dietary total fat | 101 | 81.3 | 36.5 | 146 | 88.7 | 48.6 | |
| Dietary caloric intake | 101 | 1725.1 | 627.4 | 146 | 1947 | 820.6 | P<0.023 |
| C18:2n6 | 80 | 11.9 | 2 | 123 | 10.9 | 1.9 | P<0.0005 |
| C20:2n6 | 80 | 0.23 | 0.14 | 123 | 0.19 | 0.10 | P<0.042 |
| c20:3n6 | 80 | 0.24 | 0.14 | 123 | 0.28 | 0.15 | |
| c20:4n6 | 80 | 0.31 | 0.10 | 123 | 0.40 | 0.19 | P<0.0005 |
| sum n6 fatty acids | 80 | 1.35 | 0.33 | 123 | 1.41 | 0.42 | |
| c18:3n3 | 80 | 0.49 | 0.10 | 123 | 0.48 | 0.10 | |
| c20:5n3 | 80 | 0.05 | 0.06 | 123 | 0.1 | 0.2 | P<0.015 |
| c22:5n3 | 80 | 0.23 | 0.14 | 123 | 0.27 | 0.16 | P<0.048 |
| c22:6n3 | 80 | 0.20 | 0.14 | 123 | 0.29 | 0.20 | P<0.0005 |
| sum n3 fatty acids | 80 | 0.96 | 0.30 | 123 | 1.14 | 0.41 | P<0.001 |
| Depression (Zung scale) | 73 | 33.4 | 7.6 | 92 | 30.5 | 7.4 | P<0.017 |

**Table 4.2** Anthropometric, dietary and adipose tissue fatty acid measures (mean ± standard deviation) in mildly depressed (Zung score > 40) v/s non-depressed subjects.

| | Non-depressed | | | Mildly depresssed | | | |
|---|---|---|---|---|---|---|---|
| | N | Mean | SD | N | Mean | SD | Sig |
| Age | 137 | 38.2 | 7.2 | 27 | 37.4 | 10.6 | |
| BMI | 139 | 25.7 | 3.8 | 26 | 24.8 | 3.2 | |
| Dietary total fat | 137 | 85.1 | 46.4 | 27 | 93.2 | 42.2 | |
| Dietary caloric intake | 137 | 1817.2 | 811.9 | 27 | 2015.2 | 819.6 | |
| C18:2n6 | 121 | 11.2 | 1.5 | 22 | 11.5 | 2.1 | |
| C20:2n6 | 121 | 0.21 | 0.14 | 22 | 0.20 | 0.05 | |
| c20:3n6 | 121 | 0.26 | 0.14 | 22 | 0.22 | 0.06 | |
| c20:4n6 | 121 | 0.35 | 0.15 | 22 | 0.33 | 0.1 | |
| sum n6 fatty acids | 121 | 1.37 | 0.40 | 22 | 1.30 | 0.21 | |
| c18:3n3 | 121 | 0.48 | 0.07 | 22 | 0.49 | 0.13 | |
| c20:5n3 | 121 | 0.06 | 0.07 | 22 | 0.08 | 0.06 | |
| c22:5n3 | 121 | 0.25 | 0.14 | 22 | 0.22 | 0.11 | |
| c22:6n3 | 121 | 0.26 | 0.19 | 22 | 0.17 | 0.08 | P<0.05 |
| sum n3 fatty acids | 121 | 1.04 | 0.33 | 22 | 0.97 | 0.22 | |

standard deviations of the particular variables in depressed v/s non-depressed subjects. Depressed subjects had significantly lower adipose tissue DHA ($p<0.05$). Table 4.3 depicts Pearson correlations between depression and adipose tissue fatty acids. Depression correlated inversely with adipose tissue DHA ($p<0.05$) and c20:3 n-6 ($p<0.05$). The inverse relation between depression and adipose tissue DHA was confirmed by stepwise multiple linear regression analysis. Specifically, 3% of the variability in Zung depression was significantly accounted for by adipose tissue DHA ($F=5.62$, $p<0.02$) (Table 4.4). Beta coefficient shows that adipose tissue DHA related negatively to depression.

**Table 4.3** Pearson correlations between depression and adipose tissue fatty acids.

| | Depression (Zung scale) |
|---|---|
| C18:2n6 | 0.05 |
| C20:2n6 | -0.09 |
| c20:3n6 | -0.17* |
| c20:4n6 | -0.09 |
| sum n6 fatty acids | -0.14 |
| c18:3n3 | -0.02 |
| c20:5n3 | -0.02 |
| c22:5n3 | -0.06 |
| c22:6n3 | -0.19* |
| sum n3 fatty acids | -0.14 |

*P<0.05

**Table 4.4** Stepwise multiple regression analysis with depression as the dependent variable. Independent variables were age, sex, body mass index (BMI), dietary total fat, dietary caloric intake, and adipose tissue c18:2n6, c20:2n6, c20:3n6, c20:4n6, sum n6 fatty acids, c18:3n3, c20:5n3, c22:5n3, c22:6n3, and sum n3 polyunsaturated fatty acids.

| Dependent variable | Predictor | Beta | t | P |
|---|---|---|---|---|
| *Depression (Zung scale)* | Adipose tissue c22:6n3 | -0.20 | -2.37 | <0.019 |
| | Constant | | 31.7 | <0.0005 |

## DISCUSSION

Given that adipose tissue fatty acid composition is a biomarker of long-term (1 to 3 year) or habitual dietary fat intake, [18-19] the observed negative relation between adipose tissue DHA and depression, in the present study (Tables 4.3 and 4.4), appears to indicate decreasing long-term dietary DHA intakes with increasing depression. This finding agrees with

findings of other studies indicating an inverse relation between depression and consumption of fish and n-3 polyunsaturated fatty acids (PUFA).[6-7,9-10,12-13] Fish consumption has been associated with decreases in depression prevalence, [6-7] and decreased plasma phospholipid and cholesteryl ester n-3 polyunsaturates, a marker of n-3 PUFA intake over the past few weeks, [16-17] have been observed in depressed patients as opposed to healthy controls. [9-13] However, no studies have been conducted on the relation between adipose tissue PUFA and depression. The present investigation is the first report of a negative relation between adipose tissue DHA and depression. The fact that adipose tissue DHA accounted for only 3% of the variability in depression, in the present study, may stem from the fact that the majority of the subjects (82%) were non-depressed. 18% of the sample was mildly depressed (ZSDS>40) 26 and none of the subjects exceeded the cutoff for moderate depression (ZSDS>60). [27] Nevertheless, mildly depressed subjects had significantly decreased adipose tissue DHA (-34.6%) than their non-depressed counterpart (Table 4.2). Unlike other studies, the present study failed to demonstrate a significant relation between the ratio n-6/n-3 PUFA and depression. [11]

Although the etiology of depression is yet unknown, one of the key pathophysiological features of the particular disease is a deficit in adrenergic and serotonergic neurotransmission. Reduced levels of norepinephrine and dopamine [28] as well as serotonin levels [29-30] have been reported in depression. Aside from deficits in adrenergic and serotonergic channels, depression is characterized by an upregulation of hypothalamic-pituitary-adrenal (HPA) axis and a down-regulation of its negative feedback control. [31-34] Depression is characterized, among other things, by increased secretion of adrenocorticotropin (ACTH), cortisol and growth hormone (GH) [35] and corticotropin-releasing-factor (CRF) levels, [36] and by decreased prolactin secretion. [37] According to the macrophage theory of depression, depression is the result of excessive/prolonged macrophage activation, and secretion of macrophage monokines such as IL-1, INF-α and tumor necrosis factor. [38] Indeed, depression is characterized by an immune-inflammatory response [39] or immune-inflammation markers such as increased number of activated T-cells and peripheral blood cells (i.e. B lymphocytes, CD+ T-cells, leukocytes, neutrophils and monocytes) [40-41] and increased cytokine production such as interleukin-1 (IL-1), [34,40,42-43] interleukin-2 (IL-2), [41] interleukin-6 (IL-6) [33,40,44-46] and interferon-γ (INF-γ), [47] increased number of soluble IL-2 (sIL-2R) [43,46,48] and IL-6 (sIL-6R) receptors [46,49] and elevated serum IL-1 (IL-1Ra) receptor antagonist levels. [39,50] Depression prevalence and IL-6 levels

increase with advancing age. [51] The degree or severity of depression has been reported to correlate with IL-6. [51] It has been reported that the acute phase of depression is associated with increases, while remission is associated with decreases in IL-6. 45 Positive correlations have been reported between degree or severity of depression and sIL-2R, IL-1Ra levels. [50,52]

The immune system has been reported both to be subjected to neural and neuroendocrine influence, and to exert a reciprocal regulation of neuroendocrine functions including HPA-axis activation. [53] Certain cytokines appear to exert a stimulatory effect on HPA-axis and the production of CRF, cortisol and ACTH, that are reportedly elevated in depression. For example, it has been reported that the HPA-axis activity is stimulated by cytokines such as IL-1, [34] IL-6 [33] and interferon-alpha (INF-α). [54] It has been suggested that the stimulatory effects of IL-6 on HPA-axis activity are probably mediated through its membrane receptor (IL-6R). Both IL-6 and IL-6R along with their mRNAs have been identified in many brain regions. [55] Both IL-1 and IL-6 have been reported to regulate the CRF-induced HPA-axis activation. [56] Other cytokines such as INF-α and IL-2 also have been reported to stimulate CRF release from amygdala and hypothalamus. [57] In addition to the brain, both IL-6 and its receptor are expressed on the adrenals. [58-59] IL-6 has been reported to stimulate cortisol secretion from human adrenocortical cells in vivo [60] and in vitro, [59,61] in a time- and dose-dependent fashion. [58] IL-6 stimulates the synthesis of both cortisol and ACTH. [60] In addition to IL-6, other cytokines such as INF-β, INF-γ and tumor necrosis factor also have been reported to stimulate cortisol and ACTH release. [62]

On the other hand, it has been reported that various antidepressive agents in vivo and in vitro reduce immune reactivity [63] and that tricyclic antidepressants may inhibit cytokine production from human monocytes. [64]

Moreover, administration of cytokines to cancer, multiple sclerosis and chronic hepatitis patients has led to depression, suicidal attempts and successful suicide. For example, IL-2 and INF-α administration to cancer patients led to increases in depression soon after initiation of treatment (3-5 days after). [65-66] INF-β treatment for multiple sclerosis led to increases in depression in 41% of the patients treated, within 6 months after initiation of treatment. [67] In another study, depression was induced by all 4 different types of INF-α tested, on hepatitis C patients. [68] There are consistent reports of increasing depression as a result of INF-α therapeutic regimens for cancer and viral hepatitis. [69-71] In fact, of the studies reviewed, only one study reported no effect of INF-α treatment of hepatitis, on depression. [72] In addition to depression, IL-2 and INF-α therapies, particularly INF-α

therapies, have been reported to have resulted in both suicidal attempts and successful suicide. [73-80] Often, these attempts and successful suicide are not preceded by any prior psychiatric history. [73,76,77,79] Psychiatric effects due to INF-α treatment are common and have frequently necessitated discontinuation of therapy, decreases in dose or antidepressant medication. [70]

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. [81,82] 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. [83] 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. [84-87] Given that cytokines such as IL-1, IL-2, IL-6 and TNF-α have been reported to relate positively to depression, the observed negative relation between adipose tissue DHA and depression, in the present study, may stem from the inhibiting effect of DHA on the production of the particular cytokines.

In conclusion, in agreement with findings of other studies, the observed negative relation between adipose tissue DHA and depression, in the present study, appears to indicate increasing long-term dietary DHA intakes with decreasing depression. This is the first literature report of a relation between adipose tissue DHA and depression. Given the positive relation between depression and cytokines, such as IL-1, IL-2, IL-6, INF-γ and INF-α, the observed negative relation between DHA and depression may stem from the inhibiting effect of DHA on cytokine synthesis.

**ACKNOWLEDGEMENTS**

Supported by the union of lawyers of the county of Iraklion. Other members of the research team who participated in the study include: Basil Alevizos, MD, Georgia Martimianaki and D. Kounali. We wish to thank Dr Michael Maes for the articles sent.

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# Chapter 5

# Depression and adipose n-3 polyunsaturated fatty acids in adults from Crete

Published as: Mamalakis G, Kalogeropoulos N, Andrikopoulos N, Hatzis C, Kromhout D, J Moschandreas J, Kafatos A. Depression and long chain n-3 fatty acids in adipose tissue in adults from Crete. Eur J Clin Nutr 2006; 60: 882-88.

**ABSTRACT**

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. The purpose of the present study was 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. The present investigation was 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. The study sample consisted of 130 healthy adults (59 males, 71 females) aged 22-58. The sample was a sub-sample of the Greek ApoEurope study group. Fatty acids were determined by gas chromatography in adipose tissue. Information about depression was obtained through the Zung Self-rating Depression Scale. 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 (BMI), smoking and educational level confirmed this association. 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

## INTRODUCTION

Depression constitutes the most common psychiatric disorder in adults and a major health problem in the elderly.[1,2] It has been reported that the age of onset of major depression has decreased, while its incidence has increased, the last 100 years.[3] Depression is associated with increases in all-causes mortality, particularly in men.[1]

Epidemiological studies have shown that increased consumption of fish is associated with a lower prevalence of depression.[4] 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.[5-9] 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 (C20:4n-6) to eicosapentaenoic acid (C20:5n-3) have been observed in erythocytes and serum cholesteryl esters and phospholipids of depressed patients as opposed to healthy controls.[7-9] It has been reported that plasma and serum phospholipids and cholesteryl esters reflect fatty acid intake over a few days to weeks.[10-11] 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.[12,13] Indeed, DHA has been reported to be a poor substrate for LCAT.[14] 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.[15-17]

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 to 3 year).[18,19] 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 (C18:3n-3) and depression, in a group of elderly.[20] Depressed subjects had significantly lower (-10.5%) adipose tissue C18:3n-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.[21] 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. [22] 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

### Study population

The study sample was a sub-sample of the Greek ApoEurope study group.[23] 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.[24]

### 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 (m2).

### 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.[25] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[25] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids.[25] 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).[26] The FAME were separated on a 50mx0.22mm 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).[27]

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 to 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, body mass index (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 5.1** Depression, anthropometric and adipose tissue fatty acid measures (mean ± standard deviation) in adults from Crete.

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | *Mean* | *SD* | *N* | *Mean* | *SD* | *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 |
| C18:2n-6 | 12.4 | 1.9 | 71 | 12 | 2.3 | 59 |
| C18:3n-6 | 0.15 | 0.11 | 71 | 0.13 | 0.09 | 59 |
| C20:2n-6 | 0.19 | 0.04 | 71 | 0.18 | 0.04 | 59 |
| C20:3n-6 | 0.19 | 0.06 | 71 | 0.20 | 0.06 | 59 |
| C20:4n-6 | 0.26 | 0.09 | 71 | 0.31 | 0.11 | 59 |
| C22:2n-6 | 0.04 | 0.02 | 68 | 0.04 | 0.02 | 56 |
| C22:5n-6 | 0.03 | 0.02 | 67 | 0.04 | 0.03 | 54 |
| C18:3n-3 | 0.48 | 0.08 | 71 | 0.50 | 0.12 | 59 |
| C18:4n-3 | 0.30 | 0.08 | 71 | 0.28 | 0.09 | 59 |
| C20:3n-3 | 0.04 | 0.02 | 68 | 0.05 | 0.05 | 57 |
| C20:5n-3 | 0.03 | 0.01 | 71 | 0.04 | 0.02 | 59 |
| C22:5n-3 | 0.11 | 0.04 | 71 | 0.13 | 0.04 | 59 |
| C22:6n-3 | 0.09 | 0.03 | 71 | 0.10 | 0.05 | 59 |

Table 5.1 depicts means and standard deviations of depression, anthropometric and adipose tissue fatty acid measures for the two genders. 21.6% of females 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 5.2** Spearman correlations between adipose tissue fatty acids and depression in the adults aged 22-58 from Crete.

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

Table 5.2 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, docosahexaenoic acid (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 and smoking were significantly related with depression (Table 5.3).

**Table 5.3** 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 |

## 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 5.3) agrees with studies indicating consistently higher depression rates in women as opposed to men.[28] The positive relationship between BMI and depression (table 5.3) is in line with findings of other studies.[29] For example, a prospective study of 2,123 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.[29] Indeed, obesity may lead to lower self-esteem and subsequent depression.[30] The observed positive relation between smoking and depression also agrees with findings of other studies.[31,32] 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.[33] Also, the present study failed to replicate the inverse relation between depression and educational level often reported in the literature.[34]

Given that adipose tissue fatty acid composition is a biomarker of long-term (1 to 3 year) or habitual dietary fat intake,[18,19] 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.[22] Mildly depressed subjects had 36.4% lower adipose tissue DHA levels than non-depressed subjects.[22] 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.[4] 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.[5-9] Finally, this finding is in line with findings of controlled clinical studies that have shown beneficial effects of n-3 PUFA administration on depression.[15-17] However, unlike other studies that reported elevations in arachidonic (C20:4n-6) to eicosapentaenoic acid (C20:5n-3) ratio in depression,[7-9] the present study

failed to detect any significant correlation between the particular ratios and depression (Table 5.2).

There are indications that the brain preferentially incorporates esterified over unesterified fatty acids.[35,36] It has been reported that fatty acids esterified to erythrocyte membrane phospholipids closely reflect those of neuronal membranes.[37,38] Nevertheless, adipose tissue fatty acids also may be related to brain fatty acids.[39-41] As a result of hydrolysis of adipose tissue triacylglycerols by hormone sensitive lipase and adipose triglyceride lipase, free fatty acids enter the circulation.[42-44] Non-esterified fatty acids, including DHA, supply cells, tissues, organs and brain with fatty acids.[36,45]

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.[46] 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.[47] 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.[48] Given that cytokines such as IL-1, IL-2, IL-6 and TNF-α have been reported to relate positively to depression,[49-51] 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.[52] Another study showed that DHA and arachidonic acid feeding prevented a decrease in dopaminergic and serotonergic neurotransmitters in animal frontal cortex.[53] Still, another study indicated that deficiencies in n-3 PUFA were associated with lower dopamine levels in rats.[54] Positive correlations were observed between plasma DHA and cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) concentrations, in healthy subjects.[55] CSF 5-HIAA and HVA levels reflect central concentrations of serotonin and dopamine respectively.[55] Given that depression is characterized by reduced dopamine and serotonin levels,[56,57] 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.[58,59] Dietary deficiency in n-3 PUFA has been reported to result in diminished nerve growth factor levels in rat hippocampus.[60] Another study observed decreases in neuron size in the hippocampus of rats fed DHA deficient diets.[61] Some other animal study reported that DHA protected rat hippocampal cultures from glutamate-induced cytotoxicity.[62] DHA has been credited with neuroprotective and neurotrophic properties by a number of animal studies.[63,64]

An obvious limitation of this cross-sectional study is that it can not 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.[15-17,65] 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.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Mrs Sofia Flouri and Mr Manolis Linardakis. This study was funded by the International Olive Oil Council and the DG XII of the European Union.

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# Chapter 6

# Depression and n-3 polyunsaturated fatty acids in adipose tissue and serum phospholipids in adults

Mamalakis G, M Kiriakakis M, Tsibinos G, Jansen E, Cremers H, Strien C, Hatzis C, Moschandreas J, Linardakis M, Kromhout D, Kafatos A. Depression and adipose and serum phospholipid polyunsaturated fatty acids in an adult rural population of Crete.
Submitted for publication.

**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.

## INTRODUCTION

As indicated by epidemiological studies, increased fish intake is associated with lower depression prevalence.[1,2] 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 [3-7] and occasionally higher proportions of n-6 PUFA in depressed patients as opposed to healthy controls.[5-7] 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.[8,9] 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 to 3 year).[10,11] These studies have indicated inverse relationships between depression and long-term n-3 PUFA intake,[12-15] thereby confirming the results obtained by studies using biomarkers of 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.[16] 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 as 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.

## METHODS

### Study population

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.

**Depression assessment**

Depression level was assessed through the use of the Beck Depression Inventory (BDI) and the Zung Self-rating Depression Scale (ZSRDS).[17,18] BDI and ZSRDS have been reported to be valid and reliable depression assessment tools.[19-22] The rationale for using both the BDI and the ZSRDS is that although both scales are significantly intercorrelated,[20] 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.[23] Finally, factor analytic studies indicate that often, different depression scales including the ZSRDS and the BDI, tap on different aspects or dimensions of depression.[23,24] This has led to the suggestion that depression assessments should rely on the implementation of multiple depression screening instruments.[25]

**Anthropometric Measures**

Body weight was assayed by a digital scale (Seca) with an accuracy of $\pm$ 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 and educational level were categorical variables (non-smokers=0, smokers=1), (primary school=0, at least secondary school=1).

**Adipose tissue measures**

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan.[26] 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.[16] The fatty acids have been expressed as % of the total fatty acids present in the chromatogram.

**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 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 were separated on a 100 m x 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.

**Statistical methods**

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

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).

## RESULTS

**Table 6.1** 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 |

Of all participants, 4.6% had mild to severe depression symptoms (BDI scores >= 10 and ZSRDS scores >= 50).[27] 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).[27] 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 6.1 depicts means and standard deviations of age, BMI depression and adipose tissue and serum phospholipid PUFA in the two genders and the entire group.

In univariate analysis BDI was only correlated with adipose tissue alpha-linolenic acid C18:3n-3 (ALA) ($r=-0.17$, $p<0.02$). 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 6.2).

**Table 6.2** 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 |

In univariate analysis ZSRDS correlated with adipose tissue C20:2n-6 ($r=-0.16$, $p<0.03$). This association 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 phospholipids polyunsatured fatty acids were not related to depression.

## 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.[12-15] 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.

In three previous studies individual n-3 PUFA in adipose tissue were inversely related to depression. [12,14,15] 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.[12-15] 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 to 3 year) fatty acid intake,[10,11] serum phospholipids are a biomarker of short-term intake of dietary fatty acids (1 to 2 weeks).[9] 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.[16]

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.[28] The observed positive relationship between age and depression is in line with findings from other studies.[29] Unlike other studies, however, this study failed to demonstrate the reported significant relations of depression with BMI,[30] smoking,[31] or educational level.[32]

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.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Irene Markatzi and Sofia Flouri. Funding was provided by the local government (Nomarchia) of the county of Iraklion, Crete.

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# Chapter 7

# Depression and adipose n-3 polyunsaturated fatty acids in an elderly group

Published as: Mamalakis G, Kiriakakis G, Tsibinos G, Kafatos A. Depression and adipose polyunsaturated fatty acids in the survivors of the seven countries population of Crete. Prostagl Leukotr Essent Fatty Acids 2004; 70: 495-501

**ABSTRACT**

The purpose of the present study was to investigate the relation between adipose tissue polyunsaturated fatty acids, an index of long-term or habitual fatty acid dietary intake and depression. The sample consisted of 150 elderly males from the island of Crete. The subjects were survivors of the Greek Seven Countries Study group. The mean age was 84 years. The number of subjects with complete data on all variables studied was 63. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. Depression was assessed through the use of the short form of the Geriatric Depression Scale (GDS-15). Depression correlated negatively with adipose tissue alpha-linolenic acid (C18:3n-3). Depressed subjects had significantly reduced (-10.5%) adipose tissue C18:3n-3 levels than non-depressed subjects. The observed negative relation between adipose tissue C18:3n-3 and depression, in the present study, appears to indicate increasing long-term dietary C18:3n-3 intakes with decreasing depression. This agrees with findings of other studies indicating an inverse relation between depression and consumption of fish and n-3 polyunsaturated fatty acids. This is the first literature report of a relation between adipose tissue C18:3n-3 and depression. Furthermore, this is the first report of a relation between adipose PUFA and depression in an elderly sample. Depression has been reported to be associated with elevated cytokines, such as, IL-1, IL-2, IL-6, INF-γ and INF-α. Fish oil and omega-3 fatty acids, on the other hand, have been reported to inhibit cytokine production. The observed negative relation between adipose C18:3n-3 and depression, therefore, may stem from the inhibiting effect of C18:3n-3 or its long chain metabolites on cytokine synthesis.

## INTRODUCTION

Depression constitutes one of the major health problems of the last century. [1-2] It appears that increased consumption of fish is associated with decreases in depression prevalence. [3-4] There are indications, that depletions in Docosahexaenoic acid (c22:6 n-3) (DHA), one of the long-chain PUFA in fish-oil, [5] and other long-chain n-3 PUFA may be associated with depression. Significant depletions in red blood cell membrane phospholipid DHA and n-3 long-chain PUFA, have been reported in depressed patients as opposed to healthy controls. [6] In another study, depressed patients had significant depletions of red blood cell membrane n-3 PUFA, compared to healthy controls.[7] Furthermore, red blood cell membrane levels as well as dietary intake of n-3 PUFA have been reported to have negative correlations with depression severity.[7] As indicated by another study, erythrocyte phospholipid eicosapentaenoic acid (c20:5 n-3) (EPA) correlated negatively with depression severity, in depressed patients.[8]

Besides n-3 polyunsaturates, PUFA of the n-6 family, also have been reported to relate to depression. Specifically, positive correlations have been reported between the ratio of n-6 polyunsaturated arachidonic acid (c20:4 n-6) (AA) to EPA, as well as the ratio of total n-6/n-3 PUFA in erythrocytes, and depression severity.[8] In another study, major depressed patients had significantly elevated phospholipid and cholesteryl ester AA/EPA ratios and cholesteryl ester n-6/n-3 fatty acid ratios, than minor depressed patients or healthy controls. Major depressed patients had significantly decreased serum cholesteryl ester n-3 PUFA and cholesteryl ester and phospholipid EPA, than minor depressed patients or healthy controls.[9] Finally, significantly elevated AA/EPA ratios and significantly decreased n-3 PUFA have been reported in serum cholesteryl esters and phospholipids of major depressed patients as opposed to healthy controls.[10] However, not all studies have shown decreased n-3 PUFA in depressed patients as opposed to healthy subjects. Specifically, two studies have shown significant increases rather than decreases in plasma choline phosphoglyceride and erythrocyte EPA and DHA levels in depressed patients as opposed to healthy control subjects.[11-12] Nevertheless, since plasma phospholipids and cholesteryl esters are markers of fatty acid intake of the preceding few weeks,[13-14] the decreased n-3 PUFA in depression reported by most studies, appears to reflect, in part, a corresponding reduced intake in the particular fatty acids.

Some other reason for the reported reductions of n-3 PUFA in depression, may relate to some pathophysiological features of this disease, namely inflammation and lipid peroxidation, and low zinc concentrations.[15-16] It is known that inflammatory response

system activation is associated with lipid peroxidation and reduced levels of n-3 PUFA.[16-17] Similarly, reduced levels of zinc, an inflammation marker, an antioxidant and a co-factor to the formation of desaturated and elongated products of alpha linolenic (C18:3 n-3) acid,[15] are associated with reductions in n-3 PUFA.[15] It is possible, therefore, that the reported reductions in n-3 PUFA in depression, may relate to the particular pathophysiological features of the disease.

It is worth noting that only one study used adipose tissue fatty acid measures, a biomarker of long-term (1 to 3 year) or habitual dietary fat intake. [18-19] The particular study indicated that adipose tissue DHA related negatively to depression in an adult group.[20] However, no study has as yet been conducted on the relation between adipose tissue fatty acids and depression in the elderly.

The aim of the present study was to examine the relation between depression and adipose tissue PUFA of the n-3 and n-6 families in a group of elderly males.

## METHODS

### Study population

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. The number of subjects that consented to provide adipose tissue biopsy samples was 78. Depression assessments (GDS-15) were made on 124 subjects. The number of subjects with complete data on all variables studied was 63. 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. 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. Subjects were interviewed by appointment at home or the village clinic, where they underwent a thorough physical examination and clinical test.

### 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. [21-23] GDS-15 has been standardized in elderly Greeks. [23]

**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.[24] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[24] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids. [24] 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 aim 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. [25] Briefly, 20-30 mg of fat sample were saponified with 1.0 ml NaOH in methanol and the 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 x 0.25 mm Id.SP-2560 fussed 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$^o$ C, FID 260$^o$ C, split ratio 1:4 to 1:20 (depending on the sample quantity), and oven

temperature from 140$^{o}$ C to 245$^{o}$ C with stepped temperature program: within total run time 54 min.

**Statistical methods**

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

## RESULTS

Table 7.1 depicts means and standard deviations of depression, age, anthropometric, and adipose tissue fatty acid measures in the two genders, while Table 7.2 depicts means and standard deviations of the particular variables in depressed v/s non-depressed subjects. Depressed subjects were older ($p<0.001$), had significantly lower adipose tissue alpha-linolenic acid (C18:3n-3) ($p<0.02$) and significantly higher total adipose n-6/n-3 fatty acid levels ($p<0.03$) than their non-depressed counterpart.

**Table 7.1** Depression, anthropometric, and adipose tissue fatty acid measures (mean ± standard deviation) in the elderly males.

| | N | Mean | SD |
|---|---|---|---|
| Age | 150 | 85.4 | 4.23 |
| BMI | 120 | 25.3 | 4.4 |
| GDS-15 | 124 | 4.48 | 3.68 |
| C18:2n-6 | 78 | 7.78 | 1.04 |
| C18:3n-6 | 78 | 0.05 | 0.06 |
| C18:3n-3 | 78 | 0.36 | 0.05 |
| C20:2n-6 | 78 | 0.12 | 0.03 |
| C20:3n-6 | 78 | 0.17 | 0.06 |
| C20:4n-6 | 78 | 0.31 | 0.08 |
| C20:3n-3 | 78 | 0.06 | 0.02 |
| C20:5n-3 | 78 | 0.05 | 0.02 |
| C22:3n-3 | 78 | 0.11 | 0.04 |
| C22:5n-3 | 78 | 0.15 | 0.05 |
| C22:6n-3 | 77 | 0.14 | 0.04 |
| sum n-6 fatty acids | 78 | 0.54 | 0.14 |
| sum n-3 fatty acids | 78 | 0.86 | 0.12 |
| n-6/n-3 ratio | 78 | 0.62 | 0.16 |

**Table 7.2** Anthropometric and adipose tissue fatty acid measures (mean ± standard deviation) in depressed (GDS-15 > 5) v/s non-depressed elderly males.

| | Non-depressed | | | Depressed | | | |
|---|---|---|---|---|---|---|---|
| | N | Mean | SD | N | Mean | SD | Sig |
| GDS-15 | 72 | 1.7 | 1.4 | 53 | 8.2 | 2.2 | P<0.001 |
| Age | 71 | 83.9 | 3.3 | 53 | 86.1 | 4.2 | P<0.001 |
| BMI | 60 | 25.3 | 4.0 | 39 | 26.2 | 5.3 | |
| C18:2n-6 | 43 | 8.03 | 1.1 | 25 | 7.54 | 0.9 | |
| C18:3n-6 | 43 | 0.05 | 0.01 | 25 | 0.06 | 0.1 | |
| C18:3n-3 | 43 | 0.38 | 0.05 | 25 | 0.34 | 0.04 | P<0.02 |
| C20:2n-6 | 43 | 0.12 | 0.02 | 25 | 0.12 | 0.02 | |
| C20:3n-6 | 43 | 0.17 | 0.05 | 25 | 0.19 | 0.07 | |
| C20:4n-6 | 43 | 0.31 | 0.09 | 25 | 0.32 | 0.07 | |
| C20:3n-3 | 43 | 0.05 | 0.02 | 25 | 0.06 | 0.03 | |
| C20:5n-3 | 43 | 0.05 | 0.02 | 25 | 0.05 | 0.02 | |
| C22:3n-3 | 43 | 0.11 | 0.04 | 25 | 0.12 | 0.04 | |
| C22:5n-3 | 43 | 0.14 | 0.04 | 25 | 0.15 | 0.04 | |
| C22:6n-3 | 43 | 0.15 | 0.05 | 24 | 0.14 | 0.04 | |
| sum n-6 fatty acids | 43 | 0.52 | 0.13 | 25 | 0.58 | 0.15 | |
| sum n-3 fatty acids | 43 | 0.88 | 0.12 | 25 | 0.85 | 0.12 | |
| n-6/n-3 ratio | 43 | 0.59 | 0.13 | 25 | 0.68 | 0.21 | P<0.03 |

Table 7.3 depicts Pearson correlations between depression and adipose tissue fatty acids. Depression correlated inversely with adipose tissue C18:3n-3 (r=-0.32, p<0.009) and linoleic acid c18:2 n-6 (r=-0.24, p<0.05).

**Table 7.3** Pearson correlations between depression and adipose tissue fatty acids.

| | Depression (GDS-15) |
|---|---|
| C18:2n-6 | -0.24* |
| C18:3n-6 | 0.08 |
| C18:3n-3 | -0.32** |
| C20:2n-6 | -0.14 |
| C20:3n-6 | 0.08 |
| C20:4n-6 | 0.03 |
| C20:3n-3 | 0.06 |
| C20:5n-3 | -0.02 |
| C22:3n-3 | 0.08 |
| C22:5n-3 | -0.05 |
| C22:6n-3 | -0.08 |
| sum n-6 fatty acids | 0.10 |
| sum n-3 fatty acids | -0.17 |
| n-6/n-3 ratio | 0.20 |

* P<0.05 (2-tailed)
** P<0.01 (2-tailed)

The inverse relation between depression and adipose tissue C18:3n-3 was confirmed by stepwise multiple linear regression analysis. Specifically, 7% of the variability in GDS-15 depression was significantly accounted for by adipose tissue C18:3n-3 ($F=6.02$, $p<0.02$). Beta coefficient shows that adipose tissue C18:3n-3 related negatively to depression ($B=-0.30$, $t=-2.45$, $P<0.017$).

## DISCUSSION

Given that adipose tissue fatty acid composition is a biomarker of long-term (1 to 3 year) or habitual dietary fat intake, [18-19] the observed negative relation between adipose tissue alpha-linolenic acid (C18:3n-3) and depression, in the present study (Tables 7.2 and 7.3), appears to indicate decreasing long-term dietary C18:3n-3 intakes with increasing depression. This finding agrees with findings of other studies indicating an inverse relation between depression and consumption of fish and n-3 PUFA.[3-4,6-7,9-10] Fish consumption has been associated with decreases in depression prevalence,[3-4] and decreased plasma phospholipid and cholesteryl ester n-3 PUFA, a marker of n-3 PUFA intake over the past few weeks,[13-14] have been observed in depressed patients as opposed to healthy controls.[6-10] However, only one study has been conducted on the relation between adipose tissue PUFA and depression. The particular study indicated that adipose tissue DHA related negatively to depression in an adult group.[20] The present investigation, is the first report of a negative relation between adipose tissue C18:3n-3 and depression. Furthermore, the particular study is the first literature report of a relation between adipose PUFA and depression in the elderly.

Although depressed subjects (GDS-15>5) [21,26] had significantly elevated adipose n-6/n-3 PUFA compared to their non-depressed counterpart (Table 7.2), on the whole, our analyses failed to confirm the reported significant linear positive relation between the ratio n-6/n-3 PUFA and depression. [8]

Although the etiology of depression is yet unknown, deficits in adrenergic and serotonergic neurotransmission have been reported to be implicated in the particular disorder. Depression is characterized by reduced levels of norepinephrine, dopamine [27] as well as serotonin levels, [28] and by a hypothalamic-pituitary-adrenal (HPA) axis activation [29-30] and secretion of adrenocorticotropin (ACTH), cortisol and growth hormone (GH) [31] and corticotropin-releasing-factor (CRF) levels. [32] There are indications that the HPA-axis

activation and associated hormone production in depression, is caused by cytokines, such as IL-1, IL-2, IL-6 and and interferon-alpha (INF-α). Specifically, it has been reported that the HPA-axis activity is stimulated by cytokines such as IL-1, [30] IL-6 [29] and interferon-alpha (INF-α). [33] It has been suggested that the stimulatory effects of IL-6 on HPA-axis activity are probably mediated through its membrane receptor (IL-6R). Both IL-6 and IL-6R along with their mRNAs have been identified in many brain regions. [34] Cytokines such as IL-1 and tumor necrosis factor-alpha (TNF-a), have been reported to be released by neurons and glial cells in the brain and to serve as neurotransmitters. [35] Both IL-1 and IL-6 have been reported to regulate the CRF-induced HPA-axis activation. [36] Other cytokines such as INF-α and IL-2 also have been reported to stimulate CRF release from amygdala and hypothalamus. [37] In addition to the brain, both IL-6 and its receptor are expressed on the adrenals. [38-39] IL-6 has been reported to stimulate cortisol secretion from human adrenocortical cells in vivo [40] and in vitro, [39, 41] in a time- and dose-dependent fashion. [38] IL-6 stimulates the synthesis of both cortisol and ACTH. [40] In addition to IL-6, other cytokines such as INF-β, interferon-γ (INF-γ) and tumor necrosis factor also have been reported to stimulate cortisol and ACTH release.[42]

There is ample evidence that cytokines such as IL-1, [30] IL-2, [43] IL-6 [29,44-45] and INF-γ, [46] number of soluble IL-2 (sIL-2R) [45] and IL-6 (sIL-6R) receptors [45] and serum IL-1 (IL-1Ra) receptor antagonist levels, [47] all are elevated in depression.

Furthermore, depression prevalence and IL-6 levels have been reported to increase with advancing age. [48] The degree or severity of depression has been reported to correlate with IL-6. [48] It has been reported that the acute phase of depression is associated with increases, while remission is associated with decreases in IL-6.[44] Positive correlations have been reported between degree or severity of depression and sIL-2R, IL-1Ra levels. [47,49]

Therapeutic administration of cytokines such as IL-2 [50-51], INF-α [50, 52] and INF-β[53] to cancer, multiple sclerosis and chronic hepatitis patients has led to depression. The depressive symptoms due to IL-2, INF-α or TNF-α administration, often appear soon after cytokine administration [50-51] and subside after termination of treatment. [54] Psychiatric effects due to INF-α treatment are common and have frequently necessitated discontinuation of therapy, decreases in dose or antidepressant medication. [52] In addition to depression, IL-2 and INF-α therapies, particularly INF-α therapies, have been reported to have resulted in both suicidal attempts and successful suicide. [55-56] Often, these attempts and successful suicide are not preceded by any prior psychiatric history. [55-56]

It has been reported that n-3 PUFA can suppress cytokine production. Specifically, in vitro studies have shown that EPA and DHA suppress IL-6 production by human endothelial cells. [57-58] EPA and DHA have been reported to suppress the production of IL-1, IL-2, IL-6, TNF-α and INF-γ by human lymphocytes in vitro. [59] A study on healthy male volunteers showed that dietary supplementation of C18:3n-3 and its long chain metabolites EPA and DHA[60] suppressed the production of IL-1b by monocytes.[61] Human studies have shown that dietary supplementation with C18:3n-3 has reduced the synthesis of IL-6, IL-1 and TNF-a. [62-64] Dietary supplementation with EPA and DHA has been reported to result in suppression of IL-1, IL-2, IL-6 and TNF-α production by human monocytes. [65-68] Given that cytokines such as IL-1, IL-2, IL-6 and TNF-α have been reported to relate positively to depression, the observed negative relation between adipose tissue C18:3n-3 and depression, in the present study, may stem from the inhibiting effect of C18:3n-3 or by its metabolites on the production of the particular cytokines.

In conclusion, in agreement with findings of other studies, the observed negative relation between adipose tissue C18:3n-3 and depression, in the present study, appears to indicate increasing long-term dietary C18:3n-3 intakes with decreasing depression. This is the first literature report of a relation between adipose tissue C18:3n-3 and depression. Given the positive relation between depression and cytokines, such as IL-1, IL-2, IL-6, INF-γ and INF-α, the observed negative relation between C18:3n-3 and depression may stem from the inhibiting effect of C18:3n-3 or its long chain metabolites on cytokine synthesis.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Christos Hatzis, Irene Markatzi and Sofia Flouri.

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# Chapter 8

# Depression and adipose and serum cholesteryl ester n-3 polyunsaturated fatty acids in an elderly group

Published as: Mamalakis G, Jansen E, Cremers H, Kiriakakis M, Tsibinos G, Kafatos A. Depression and adipose and serum cholesteryl ester polyunsaturated fatty acids in the survivors of the seven countries study population of Crete. Eur J Clin Nutr 2006; 60: 1016-23.

**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 has the closest relationship to depression. The objective of the present study was to examine the relationship of depression with both adipose tissue and serum cholesteryl ester 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 elderly men from the island of Crete. The study sample consisted of 150 males, aged 80-96. The subjects were survivors of the Greek Seven Countries Study group. Subjects were examined by the Preventive Medicine and Nutrition Clinic of the University of Crete. 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). 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. 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 polyunsaturated fatty acids to the brain.

## INTRODUCTION

Epidemiological studies have shown that increased consumption of fish is associated with decreases in depression prevalence.[1-3] 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.[4-8] 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.[6-8] Nevertheless, given that plasma and serum phospholipids and cholesteryl esters reflect fatty acid intake over a few-day to few-week period,[9,10] the decreased n-3 polyunsaturated fatty acids 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.[11-13] The adipose tissue composition is a biomarker of long-term or habitual dietary fat intake (1 to 3 year).[14,15] Two of the three studies examining the relationship between adipose tissue PUFA and depression reported negative relations between n-3 PUFA and depression.[11,12]

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

### Study population

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. 78 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 polyunsaturated fatty acids in the particular elderly group.[12] 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.[16-18] GDS-15 has been standardized in elderly Greeks.[18]

### 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 (m2).

**Adipose tissue measures**

Buttock subcutaneous tissue samples were collected by aspiration, using the method described by Beynen and Katan.[19] The particular method has been reported to be rapid and safe, and to cause no more discomfort than a routine venipuncture.[19] Buttock adipose tissue samples can be safely stored for up to 1.5 year without changes in the component fatty acids.[19] 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.[20] Briefly, 20-30 mg of fat sample were saponified with 1.0 ml NaOH in methanol and the 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 m x 0.25 mm Id.SP-2560 fussed 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 to 1:20 (depending on the sample quantity), and oven temperature from 140° C to 245° C with stepped temperature program: within total run time 54 min. The fatty acids have been expressed as % of the total fatty acids present in the chromatogram.

**Serum cholesteryl ester fatty acid measures**

200 μl serum 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 NH2 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 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 were separated on a 100 x 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.

**Statistical methods**

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

## RESULTS

Table 8.1 depicts means and standard deviations 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). Spearman 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.

**Table 8.1** Serum cholesteryl ester and adipose tissue fatty acids (mean ± standard deviation) in the male survivors of the Seven Countries Study population of Crete.

| | | *Serum cholesteryl ester* | | | *Adipose tissue* | | |
|---|---|---|---|---|---|---|---|
| | | N | Mean | SD | N | Mean | SD |
| *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 |

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 8.1).

**Figure 8.1** Scatterplot of depression against adipose C18:3n-3

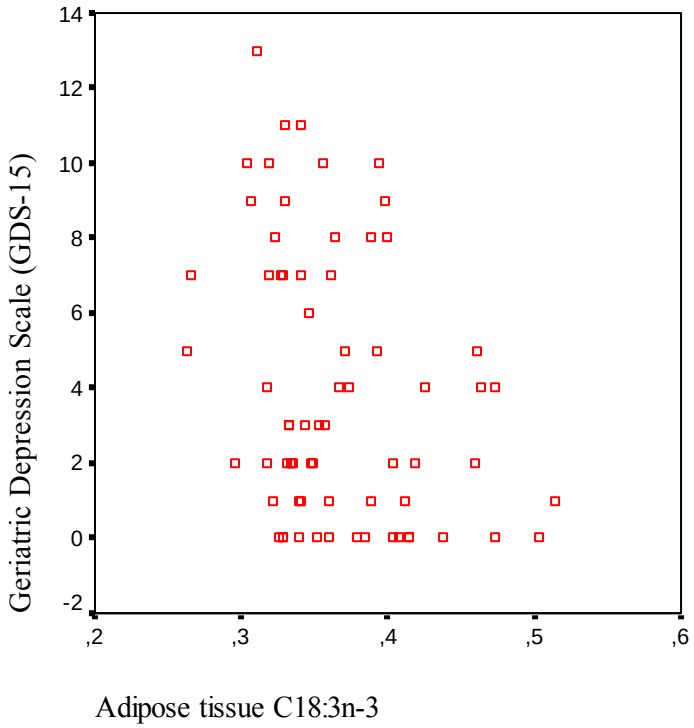


Table 8.2 depicts means and standard deviations for the serum cholesteryl ester and adipose tissue PUFA in depressed v/s non-depressed subjects. Depressed subjects were older than their non-depressed counterpart ($p<0.001$). Depressed subjects had significantly lower adipose tissue alpha-linolenic acid (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.

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

**Table 8.2** Anthropometric, serum cholesteryl ester and adipose tissue fatty acid measures (mean ± standard deviation) in depressed v/s non-depressed survivors of the Seven Countries Study population of Crete.

| | | Non-depressed (GDS-15 <= 5) | | | Depressed (GDS-15 > 5) | | | |
|---|---|---|---|---|---|---|---|---|
| | | N | Mean | SD | N | Mean | SD | Sig |
| *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 | |

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 ($B=-0.31$, $t=-2.6$, $P<0.01$).

## 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 >=4).[21] By contrast, 51.6% of the survivors of the Greek Seven Countries Study group were depressed (GDS-15 >=4). Also, in a representative study of 14217 people aged 75 and over in the UK, 7.7% of the 80-84 year age group were depressed (GDS-15 >=6).[22] The corresponding proportion of depression (GDS-15 >=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 AA and DHA on prostaglandin E-2 ($PGE_2$) production. AA is the immediate precursor of $PGE_2$.[23] On the other hand, DHA inhibits the formation of $PGE_2$.[24-26] It has been proposed that elevated levels of $PGE_2$ are implicated in depression.[26-33] 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.[7,8] 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.[6-8] 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.[34,35] Animal studies have indicated that n-3 PUFA, including DHA, exert neuroprotective effects in the hippocampus,[36-38] whereas AA and its cyclooxygenase and lipogygenase metabolites exert opposite effects.[39-42]

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 8.2). 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 one the 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.[12] However, serum cholesteryl ester fatty acid measures had not been included in the statistical analysis at that time.[12] The adipose tissue is a biomarker of long-term (1 to 3 year) or habitual dietary fat intake.[14,15] On the other hand, serum cholesteryl esters is a biomarker of fatty acid intake of the preceding 1 to 2 weeks.[10] 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 polyunsaturated fatty acids 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.[43-45] The speed of recuperation from PUFA depletion was slower in the brain compared to other tissues.[43] It has been reported that, in animals, total recovery of brain PUFA takes several weeks to complete.[43-47] It is estimated that there is a 0.3% daily replacement rate for arachidonic acid in the human brain.[48] 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 TNF-alpha.[49-51] 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.[52-54] 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.[55,56] 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.[57-59] 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 PGE2 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 polyunsaturated fatty acids 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.

**ACKNOWLEDGEMENTS**

We would like to acknowledge the invaluable contribution of: Christos Hatzis, Irene Markatzi and Sofia Flouri.

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# Chapter 9

# General Discussion

The aim of this thesis was to gain more knowledge on the relationship of depression with polyunsaturated fatty acids (PUFA) of the n-3 series. The studies presented in this thesis attempt to answer the question whether depression is associated with long-term or habitual n-3 PUFA intake in different age-groups. A small number of studies have investigated the relationship of depression with objective indicators / biomarkers of short-term n-3 PUFA intake. However, no studies have as yet investigated the extent to which depression relates to biomarkers of long-term n-3 PUFA intake. The studies of this thesis attempt to shed light on the relationship between depression and n-3 PUFA in the adipose tissue, a biomarker of long-term or habitual n-3 PUFA intake. The study populations in this thesis were comprised of generally healthy adolescent, adult and elderly subjects.

**MAIN FINDINGS**

Significant relationships between individual n-3 PUFA and depression were not manifested in our adolescent group (chapter 2). In the same adolescent group, inclusion of serum adiponectin along with the rest of the covariates in the statistical analysis, yielded a significant inverse relationship between scores on the Beck Depression Inventory (BDI) and adipose EPA (chapter 3).

A significant inverse correlation between depression and adipose tissue DHA was observed in one of our adult groups. A comparison of mildly depressed adults and non-depressed ones indicated significantly lower (-34.6%) adipose tissue DHA levels in the former sub-group. The observed relationship could not be attributed to age, gender, body mass index, or educational level (chapter 4). The inverse relationship between depression and adipose tissue DHA was also observed in a second adult group. The observed relationship was not due to age, gender, body mass index, smoking or educational level (chapter 5). In another adult group, depression was not significantly associated with adipose tissue n-3 PUFA levels (chapter 6).

A significant inverse relationship between depression and adipose tissue ALA levels was observed in an elderly group. Depression correlated negatively with adipose tissue ALA levels in the elderly subjects of the Seven Countries Study population of Crete. Depressed males had significantly lowered (-10.5%) adipose ALA levels than non-depressed ones (chapter 7). Finally, inclusion of serum cholesteryl ester fatty acids in the statistical analysis did not change the results obtained. Namely, the observed significant relationship between depression and adipose ALA levels still persisted (chapter 8).

An overview of the main findings of this thesis is presented in table 9.1.

**Table 9.1** Main findings of the studies described in this thesis.

| Age-group | Depression | Association? | Adipose n-3 fatty acid | Part Corr | Chapter |
|---|---|---|---|---|---|
| Adolescents | BDI, CES-D | No | | | 2 |
| Adolescents | BDI | Yes | EPA | -0.24* | 3 |
| | CES-D | No | | | 3 |
| Adults | ZSRDS | Yes | DHA | -0.20* | 4 |
| Adults | ZSRDS | Yes | DHA | -0.23** | 5 |
| Adults | BDI, ZSRDS | No | | | 6 |
| Elderly | GDS-15 | Yes | ALA | -0.30* | 7 |

*$p<0.05$, **$p<0.01$
Part Corr=partial correlation between adipose n-3 fatty acid and depression**,** BDI=Beck Depression Inventory, CES-D=Center for Epidemiologic Studies Depression Scale, ZSRDS=Zung Self-rating Depression Scale, GDS-15=Geriatric Depression Scale (short form), EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, ALA=alpha-linolenic acid

## METHODOLOGICAL CONSIDERATIONS

The research of this thesis falls under the realm of what it is referred to as descriptive or observational quantitative research. Typically, this type of research is concerned with either identifying the characteristics or factors associated with the occurrence of a target outcome such a disease or a medical condition, or exploring for possible correlations between the particular factors and the target outcome. In either case, this type of research does not alter or modify the situation but rather examines the situation as it is. The studies of this thesis are cross-sectional studies. These studies, also known as surveys, aim at gathering information about a large population by surveying a segment or a cross-section of that population. Often, cross-sectional studies recruit different age-groups at once, in spite the different metabolic features inherent in those groups. This can be a source of confounding, referred to as a cohort effect. However, given that different age-groups were studied separately in this thesis, it is unlikely that the results presented here have been constrained by this source of bias.

Cross-sectional studies collect information from one or more groups of subjects through interviews or questionnaires. The cardinal feature of cross-sectional research is that it provides a snapshot of the population at one particular point in time. Unlike cohort or

experimental studies, for example, cross-sectional studies do not employ follow-up measures. Furthermore, cross-sectional studies do not manipulate risk factors or exposures in an attempt to observe for possible changes in the target outcomes or disease states. Given that exposure and the outcome are ascertained at the same time, or measures of risk factors or exposures and outcomes (i.e. disease status) are assessed concurrently in cross-sectional studies, it is often not possible to disentangle the exposure from the outcome and ascertain which came first. An exception is long-lasting or pervasive exposures / traits such as sex, race and certain constitutional / genetic traits that have beyond doubt preceded the outcome. A major limitation of cross-sectional studies, in other words, is that it is often not possible to establish a cause and effect relationship between exposures or risk factors and the target outcome or disease status. The research of this thesis is correlational research. Correlational research is particularly useful when dealing with complex relationships involving large numbers of variables. Often, correlational research is an invaluable tool for exploring relationships among variables that are not manipulated, as is the case in cross-sectional studies. Two variables correlate when they co-vary in a predictable fashion. The correlation coefficient r, indicates how strong a relationship is between two variables as well as the direction of that relationship.

Validity is a criterion for assessing the quality of research. Questions raised to the quality of the methodology applied or study design implemented or the accuracy of findings is a threat to validity. All studies are subject to validity threats. There are two kinds of validity, internal and external validity. Any factor within the study that allows for a different explanation for the findings, other than the one proposed, is a threat to internal validity. Conversely, any factor that limits the extrapolation or applicability of research findings to the general population constitutes a threat to external validity. Threats to internal validity constitute threats to external validity as well, but not the other way around. The correlation coefficient as an estimate or measure of association in this thesis can either be an accurate estimate or it could up to a certain extent be reflecting bias. A biased estimate is one that over- or underestimates the quantity being measured. Statistical associations, significant correlations in particular, do not necessarily denote cause and effect relations. There are three types of associations: spurious or illusory (resulting from selection bias, measurement bias or chance), indirect (stemming from confounding) and causal. The first two of these associations, namely spurious and indirect associations, threaten the internal and also the external validity of a study and will be dealt with in detail in the following two sub-

sections. The possibility that there may be a causal relationship between n-3 PUFA intake and depression will be dealt with in the Evidence in favor of causality sub-section, while issues concerning the external validity and clinical significance of findings will be dealt with in the last sub-section of the Methodological considerations section.

***Spurious or illusory correlations***

The mere fact that our participants were volunteers, may have constituted a self-selection bias (consenter bias). It has been reported that consenters or volunteer participants in health surveys are healthier, more educated, younger, and of higher socioeconomic status than non-consenters.[1] It is possible that our study samples that consisted of predominantly non-depressed subjects were biased in favour of including non-depressed rather than depressed participants. Moreover, a number of volunteer participants surrendered blank or partially complete depression questionnaires and were thus excluded from the study (non-respondent or non-response bias). Often, in cross-sectional studies, due to the large number of parameters assessed and questionnaires administered, fatigue occurs and subsequent inaccurate responding or failure to respond results. It is possible that this response failure has affected both the depressed and the non-depressed participants alike (self-selection bias due to random, non-systematic or non-differential error). However, given that depressed individuals tend to be fatigued (one of the symptoms of depression) and drop-out, fatigue may have had a more pronounced influence (differential effect) upon the depressed than the non-depressed volunteers. It is possible, in other words, that depressed volunteers were more likely than the non-depressed ones to yield blank or partially complete depression tests and to be excluded from the study (self selection bias due to non-random, systematic or differential error).

It may sound paradoxical that in this thesis depression was studied in healthy non-clinically depressed groups. Depression is not an all-or-non phenomenon. Rather, depression is a continuum operationalized as the degree to which certain somatic and behavioral symptoms and subjective feelings characteristic of depression are reported by the study participants. In reality, few if any at all people are totally free of all depressive symptomatology tapped on by depression inventories. In fact, obtaining a zero score on a depression inventory may be an indication that one may be deliberately lying. Nevertheless, on the basis of the scores obtained, subjects can be classified as non-depressed, mildly depressed or severely

depressed. In most cases, depression has been treated as a continuum or as a continous measure in this thesis.

The magnitude of a correlation is always range-dependent or contingent upon the range of values of the variables correlated. Restriction of range in the values of either the predictor or the outcome measure, in most occasions results in deflated association estimates.[2] However, in a few instances even inflated association estimates could result.[2] The lack of mildly to severely depressed or clinically depressed subjects in this thesis may have been a source of bias due to the restriction of range of values in the outcome measure (range restriction bias or scale attenuation effect). This may have led to a lower correlation between depression and n-3 PUFA than the one that might have been obtained had depressed participants been included in the thesis.

Adipose tissue measures are seldom implemented in epidemiologic studies due to difficulties in obtaining the samples.[3,4] Due to the limited number of studies and scarcity of data, therefore, there is no normative data or detailed information on the distribution and range values of the different adipose n-3 fatty acids across different age groups. However, it appears that there may have been a restriction of range in C18:3n-3 (ALA) and C22:6n-3 (DHA), the n-3 fatty acids that in this thesis related significantly to depression. The coefficient of variation (Cv) can be used an estimate of the value range or an index of the data scatter with respect to the mean. Cv, sometimes referred to as the relative standard deviation, is particularly useful in comparing different samples with unequal arithmetic means. Cv is the ratio of the standard deviation divided by the arithmetic mean, multiplied by one hundred (Cv=SD/mean*100). It appears that ALA and DHA can assume values higher than those observed for some age-groups in this thesis. For example, the mean and Cv for ALA in our elderly subjects were 0.36 and 14% respectively. By contrast, the mean and Cv for ALA in Cypriot children were 0.56 and 25% respectively.[5] Also, the corresponding values in Costa Rican adults were 0.62 and 29% respectively.[6] With respect to DHA values, it appears that one of the adult groups of this thesis was range restricted. The mean and Cv values for that group were 0.10 and 50% for men, and 0.09 and 33% for women respectively (Chapter 5). By contrast, the corresponding values for another adult group in this thesis, were 0.29 and 60% for men, and 0.20 and 70% for women respectively (Chapter 4). It appears that with reference to DHA, the former group was range restricted compared to the later one. It is possible therefore, that restriction of value range in ALA

and DHA in this thesis may have resulted in decreased association estimates between depression and the particular fatty acids. This is a potential threat to the precision of the observed estimates of association between depression and the particular n-3 fatty acids.

Nevertheless, in an attempt to accentuate association estimates between depression and n-3 PUFA or help highlight differences in n-3 PUFA between depressed and non-depressed participants, wherever possible, subjects were grouped as non-depressed versus mildly depressed. In some instances, when the number of the mildly depressed subjects was very small, the study participants were divided by the median into less-depressed versus more-depressed sub-groups.

Whatever the case, for the most part, the study participants of this thesis were non-depressed. In other words, it is possible that due to self-selection bias on the one hand and flaws in the study design of this thesis and associated range restriction bias on the other, the observed association between depression and n-3 PUFA may be an underestimation of the true relationship between the particular measures. As a consequence, these two sources of bias limit the internal validity, as well as the applicability of our findings to the general population or the external validity of the findings presented.

Besides self selection bias, it is possible that errors regarding the measurement of the different variables such as exposures, confounders and outcome measures may have had an influence on the observed association between depression and n-3 PUFA (measurement or information bias). Data entry errors often occur in studies, particularly when a large number of factors are measured. Such errors tend to uniformly affect the different groups involved (i.e. the more and the less depressed) and as such they constitute a source of non-differential or non-systematic error. Generally, as a result of this error, the association between the exposure and the outcome measures gets weakened (dilution towards the null). Nevertheless, care was taken to detect and correct unusual data entries. This type of error must not have affected the fatty acid variables as data entry for the different fatty acids was not done manually but was computerized (i.e. the different peaks were automatically transformed into a numeric form and fed into the computer as a file). Often, ambiguities in questionnaire items, is a source of non-systematic error. However, our findings were not influenced by this form of bias as the instruments used to assess depression have been standardized and their psychometric properties have been established (reliability and validity indices). The reliability of the depression inventories used safeguards against a

potential non-differential measurement bias, whereas their validity demonstrates that depression is the underlying construct assessed thereby ascertaining the precision of the particular measure.

The use of dietary intake measures such as dietary history or food-frequency questionnaires or estimated or weighed food records is flawed with bias stemming from inaccurate recall and reporting, changes in seasonal eating patterns, inherent limitations in the use of the food consumption tables used to assess intake of individual fatty acids, and over- or under-reporting of foods regarded as more socially acceptable or less socially acceptable respectively. The use of the adipose tissue, on the other hand, does not have such shortcomings. Actually, the adipose tissue and other biomarkers of fatty acid intake such as serum triacylglycerols, cholesteryl esters and phospholipids and erythrocyte membranes are often used to validate dietary questionnaires. However, serum triacylglycerol, cholesteryl ester and phospholipid and erythrocyte membrane fatty acid composition reflects fatty acid intake of the preceding few hours to several weeks.[7] The adipose tissue fatty acid composition, on the other hand, reflects fatty acid intake of the preceding 2 to 3 years and as such it is preferred over the other biomarkers as a measure of long-term fatty acid intake. Owing to its slow turnover [3,8,9] and insensitivity to acute disease,[10,11] adipose tissue is perhaps an ideal medium for the study of long-term fatty acid intake. The use of the adipose tissue as a biomarker of long-term fatty acid intake may be especially suited for the study of diseases that can have a long course and duration such as depression. However, some weakness in using the adipose tissue measures is that the different individual fatty acids assessed reflect relative or qualitative (% of the total fatty acids in the chromatogram) rather than absolute or quantitative (e.g. mg) fatty acid intake.

***Confounding***

The failure to account or adjust for the effect of one or more variables associated with both the exposure and the outcome leads to a systematic error referred to as confounding. A confounder is a factor that correlates both with the exposure and the outcome and explains some or all of the association between the two. The way to control for or eliminate confounding in cross-sectional/correlational studies is via including all potential confounders as covariates in a multivariate statistical analysis. The multivariate statistical models used in this thesis were multiple linear regressions and logistic regressions. Any

variable that correlates both with n-3 PUFA and depression is a potential confounder to our findings.

Literature review shows that a number of factors such as pessimism, self-esteem, interpersonal competence, attributional style, emotionality, neuroticism, harm avoidance, stress, social isolation and dysphoria, are all correlated with depression.[12-16] However, to the best of our knowledge, no studies have as yet investigated possible relationships / correlations between those factors and n-3 PUFA. Consequently it is not known whether these factors could be potential confounders of the relationship between n-3 PUFA and depression and were not included as covariates in the multivariate analyses of this thesis.

On the other hand, a number of factors has been reported to be associated with adipose n-3 PUFA. Although n-3 PUFA are not endogenously synthesized but derive exclusively from dietary sources, adipose n-3 PUFA levels reflect, up to a certain extent, the effect of adipogenic and adipolytic factors. The mechanism of uptake as well as selective mobilization of fatty acids from adipocytes is not yet known in detail.[17] Nevertheless, it has been reported that the deposition and the mobilization of adipose tissue fatty acids are affected by lipoprotein lipase, hormone-sensitive lipase and adipose triglyceride lipase. Furthermore, long-chain n-3 PUFA result from desaturation and elongation of their shorter-chain precursors by desaturases and elongases. To the best of our knowledge, no studies have yet been conducted on the relationship between depression and lipoprotein lipase, hormone-sensitive lipase, adipose triglyceride lipase, desaturases and elongases. Therefore, it is not known whether these factors could qualify as potential confounders to the relationship between n-3 PUFA and depression and were not included as covariates in our multivariate analyses.

However, a number of factors have been reported to be correlated to both n-3 PUFA and depression. These include monoamines (i.e. serotonin and dopamine), pro-inflammatory cytokines (i.e. IL-1, IL-2, IL-6, TNF-a), and prostaglandins (i.e. $PGE_2$) In addition, besides relating to depression, there are indications that anxiety may also relate to n-3 PUFA.[18-20] The failure to assess these factors and include them as confounders in our analyses must have beyond doubt affected the precision of the observed association estimates between depression and n-3 PUFA and is thus one of the limitations of this thesis. The covariates assessed in this thesis were global or universal predictors (established predictors/robust predictors) of depression such as age, gender and BMI.

***External validity***

The different age groups in this thesis consist of healthy volunteer participants and do not necessarily represent their respective age groups of the general population. It has been reported that depression can be observed in as young as 6-year old children.[21] Children were not among the study groups of this thesis. The reasons were difficulties in obtaining adipose tissue samples and assessing depression in this age-group.[22] The fact that children have not been included in our studies, is another factor that limits the generalizability of our findings. Whether the associations between depression and n-3 PUFA observed in adolescents, in adults and elderly in the present thesis, also hold true in children is not known and deserves further investigation.

In this thesis we adjusted for the effect of gender. However, the possibility of effect modification by gender can not be ruled out. In fact, a cross-sectional study indicated a significant inverse association between fish consumption frequency and depression in women, but not in men.[23] Due to the relatively large number of predictors and limitations in the size of the study groups recruited, it has not been possible to perform separate statistical analyses for each gender. The fact that gender has not been treated as a potential effect modifier in this thesis, limits the external validity of our findings. Future studies that will examine for possible associations between adipose n-3 PUFA and depression in each gender separately, are therefore needed.

## IS THE ASSOCIATION BETWEEN N-3 FATTY ACIDS AND DEPRESSION CAUSAL?

A number of criteria help identify causal associations or reinforce the evidence in favor of causality. These include temporal sequence, strength of association, biological gradient (dose-response relationship), consistency of association, experimental evidence, reversibility, biological plausibility, and specificity of association.[24-28] Temporal sequence refers to the extent exposure precedes the outcome or disease state. Strength of association denotes how strongly an exposure and an outcome are related. Dose-response relationship denotes that increasing levels in the exposure factor are associated with correspondingly increasing levels in the outcome measure (e.g. symptom severity, disease risk) Consistency

of association refers to the extent to which an observed relationship between an exposure and an outcome repeats itself under different study / test conditions. Experimental evidence refers to the extent to which the relationship between the exposure and the outcome has been confirmed experimentally. Reversibility refers to the extent to which reduction or removal of the exposure is associated with a corresponding reduction or remission of disease (e.g. symptom reduction / resolution). Biological plausibility refers to the extent to which a relationship between an exposure and an outcome is backed by biological evidence or that specific biological mechanisms explain the observed relationship. Specificity of association refers to the extent to which the exposure leads to one specific outcome or disease state rather than a wide range of outcomes or diseases.

It has been suggested that consistency, strength of association, dose-response, plausibility and temporality are perhaps the most important causal criteria in nutritional epidemiology.[29] However, it is our contention that in addition to these, the criteria of experimental evidence and reversibility are also of key importance in making inferences about causality. In the next sections, an attempt is being made to examine whether our findings and those of others satisfy the criteria of temporality, strength of association, dose-response relationship, consistency of association, experimental evidence, reversibility and biological plausibility, in favor of causality

***1. Temporality***

Cross-sectional studies like ours, can not establish a cause and effect relationship between exposures or risk factors and the target outcome or disease state. The reason is that risk factors and disease state are assessed concurrently at one given point in time. As a consequence, it can not be ascertained whether risk factors or exposures have preceded the outcome or disease. It appears that this must not have been the case with adipose tissue fatty acids. Although adipose tissue samples and depression measures were obtained concurrently, adipose tissue fatty acid measures have not been reflecting the type of fat consumed at the time of sampling, but rather that of the preceding 2 to 3 year period.

On the other hand, with the exception of the Zung depression scale that assesses general depression (i.e. symptoms experienced generally or over an unidentified time frame), most of the depression scales administered (i.e. BDI, CES-D and GDS-15) attempt to assess current levels of depressive symptomatology. BDI, CES-D and GDS-15, in other words,

instruct respondents to indicate the extent to which they have experienced a number of depressive symptoms during the preceding one week. Actually, depression assessment instruments like BDI, CES-D and GDS-15, are often used to detect changes in depression due to, for example, some therapeutic intervention over brief time periods (e.g. weeks / months). It would appear, therefore, that in our study subjects dietary intake of n-3 PUFA must have preceded current depressive symptomatology and not the other way around. This would be an indication that the criterion of temporal sequence has been met in our studies or that n-3 PUFA intake has not been an epiphenomenon of depression. However, this need not necessarily be the case. The reason is that depression is often a recurrent and a long-lasting or chronic illness. It should be born in mind, that in our studies we have investigated degree of depression symptomatology, for the most part, in non-depressed subjects. Nevertheless, the possibility that the degree of depressive symptomatology observed in our subjects has been an enduring and long-lasting personality attribute (trait depression) rather than a transient one (state depression) can not be ruled out. In fact, it has been reported that BDI, CES-D and Zung depression scale in addition to measuring state depression can also, perhaps even better so, measure trait depression.[30] This arouses suspicions that the depression levels observed in our subjects may have been a long-lasting personality trait and as such it may have not been a consequent to fatty acid intake.

However, even if fatty acid consumption has preceded depression, this would by no means indicate that adipose n-3 PUFA per se are necessarily related causally to depression. The reason is that in addition to diet, the levels of adipose n-3 PUFA are determined also by a number of known (i.e. lipoprotein lipase, hormone-sensitive lipase, adipose triglyceride lipase, desaturases and elongases) as well as unknown metabolic factors. It is possible, therefore, that some known or even some unidentified metabolic factor may have been responsible for the apparent relationship between adipose n-3 PUFA and depression. In other words, it is possible that some biological / biochemical factor has been responsible for both elevating / causing depression, and decreasing adipose n-3 PUFA.

Nevertheless, support to the temporal sequence criterion is provided also by controlled clinical trials of n-3 PUFA administration on depressed patients.[31-33] These studies showed that n-3 PUFA administration was accompanied by significant decreases in depression, thereby suggesting that the observed associations between n-3 PUFA and depression in this thesis, may not be an epiphenomenon of depression.

### *2. Strength of association*

Often, statistical significance can be reached in the absence of a clinically or biologically meaningful difference. Often, a highly statistically significant difference (finding) is of no biological or clinical significance. Traditionally, clinical significance is discussed with reference to some notable therapeutic response as a result of a therapeutic intervention.[34,35] In the absence of a follow-up or therapeutic intervention component in cross-sectional/correlational studies, however, the issue of clinical significance can not be addressed. Instead, the magnitude of association estimates can be used as index of the strength of the association between an exposure and an outcome measure. One association estimate in multiple regression analysis is the adjusted $R^2$ or the amount of the variability in the outcome measure accounted for by the exposure factor, adjusted to the presence of the rest of the confounders in the model. Examination of adjusted $R^2$ in our studies, shows that only a small portion (< 7%) of the variability in depression is accounted for by n-3 PUFA. Some other association estimate can be standardized regression coefficients (beta coefficients). Beta coefficients are partial regression coefficients expressed as standardized Z scores. Beta coefficients show how important each independent variable is in predicting the outcome measure. In other words, beta coefficients show how strongly each independent variable is related to the dependent measure. Examination of beta coefficients in the studies of this thesis, shows that n-3 PUFA are equally good predictors of depression as established and robust predictors of depression such as age, gender and BMI. The importance of n-3 PUFA in predicting degree of depression in healthy humans, is further highlighted by the fact that the particular fatty acids were significant predictors of depression across all different age groups studied. Furthermore, the fact that the relationship between depression and n-3 PUFA became manifest irrespective of the depression assessment instrument (scale / inventory) used, may be another indication of the strength of this relationship.

### *3. Dose-response relationship*

For the most part, in the absence of clinically depressed subjects in our study groups, depression has been treated as a continuum rather than an all-or-none entity. Degree of depression was found to vary linearly as a function of the different levels in adipose n-3 PUFA. Clearly, the higher the n-3 PUFA level the lower the depression level, a finding that derived from a multitude of values along the depression continuum and that of adipose n-3

PUFA. Beyond doubt, there was a dose-response relationship between n-3 PUFA and degree of depression in our study groups. The observed dose-response relationship is supported by two studies involving depressed patients. Depression severity within depressed patients, was inversely related to EPA and total n-3 PUFA levels.[36,37] This apparent dose-response relationship between n-3 PUFA and degree of depression both in non-depressed as well as depressed subjects, further reinforces the evidence in favor of causality.

***4. Consistency of association***

Our findings of an inverse relationship between depression and adipose n-3 PUFA are supported by those of other studies, albeit studies that have used different research paradigms and settings and study groups. Many of these studies were conducted on depressed patients and healthy controls. By contrast, our studies involved predominantly non-depressed subjects. Furthermore, many of these studies consisted of adults, whereas our studies consisted of adolescent, adult and elderly subjects. Some of these studies have used dietary intake measures such as dietary history or food frequency questionnaires, whereas other studies have used objective indicators (biomarkers) of short-term (few days to few months) n-3 PUFA intake. These biomarkers were red blood cell membrane phospholipids, plasma and serum phospholipids and plasma and serum cholesteryl esters. Unlike our studies, however, no studies have implemented biomarkers of long-term (2-3 years) n-3 PUFA intake.

For example, one study reported a inverse correlation between per capita fish consumption and annual depression prevalence in nine countries.[38] However, it appears questionable whether per capita fish consumption is as an estimate of long-term fish intake. Furthermore, it did not become apparent which particular n-3 PUFA (i.e. DHA v/s EPA) contributed more to the observed correlation. Another ecological study observed strong inverse correlations between postpartum depression and fish consumption and DHA levels in breast milk, in 22 and 16 countries respectively.[39]

Two cross-sectional studies have indicated inverse relationships between fish consumption and depressive symptoms.[23,40] It must be noted though, that in these studies, dietary intakes were assessed by fish-frequency questions and food-frequency questionnaires. The particular dietary intake estimation methods, however, are handicapped by bias due to inaccurate recall and reporting and changes in daily and seasonal eating patterns.

Moreover, in these studies, there were no repeated dietary assessments over extended time periods. In light of these, it is unlikely that the estimated n-3 PUFA intake reflected long-term intakes. Another cross-sectional study involved 771 newly diagnosed lung cancer patients. Patients with depression did not differ significantly from those without depression in EPA or DHA intakes. However, significant inverse relationships were observed between dietary intakes of ALA and total n-3 PUFA and depression.[41] The fact that depressed patients differed significantly from non-depressed ones on ALA but not EPA or DHA intakes suggests that the relationship of depression with ALA could not be mediated by EPA or DHA. This raises the possibility of an independent effect of ALA on depression. However, this is not necessarily the case, as dietary assessments were made by food-frequency questionnaires rather than biomarkers of fatty acid intake.

A case-control study reported that major depressed patients had significantly lower serum cholesteryl ester ALA than healthy control subjects. In addition to ALA, depressed patients also had significantly lower serum cholesteryl ester and phospholipid EPA in relation to healthy controls. These findings could not be attributed to reduced food intake or weight loss on the part of the depressed patients.[42] In addition, the fact that depressed patients did not differ significantly from non-depressed ones in DHA, indicates that the relationship of depression with ALA and EPA could not be mediated by DHA. The second case-control study found significantly lower ALA and EPA levels in the serum cholesteryl esters of major depressed patients as opposed to control subjects.[43] In addition, the patient group had significantly lower serum phospholipid EPA and DPA (docosapentaenoic acid, C22:5n-3) levels than the healthy control group. Much like the previous study, these findings were not due to a lower food consumption of the depressed patients. Also, similar to the previous study, the fact that depressed patients did not differ significantly from non-depressed ones in DHA levels indicates that the relationship of depression with ALA and EPA could not be mediated by DHA.

A study involving 100 suicide-attempters and 100 control patients injured by accidents, reported significantly lower EPA in the red blood cell phospholipids of suicide-attempters as opposed to the control patients. A significant eightfold difference in suicide attempt risk was observed between the lowest and the highest quartiles of red blood cell phospholipid EPA levels.[44] Yet, another case-control study indicated that both EPA and DHA were lower in the red blood cell membrane phospholipids of depressed patients compared to those of normal controls.[37] This finding was not due to reduced caloric intake on the part of the

depressed patients. As indicated in a study of patients recovering from acute coronary syndromes (ACS), depressed patients had significantly lower serum DHA and total n-3 PUFA levels than non-depressed patients, approximately 2 months after ACS.[45] Finally, another case-control study reported significantly lower DHA and total n-3 PUFA levels in the erythrocyte membrane phospholipids of depressed patients as opposed to healthy controls.[46]

Two cohort studies have shown significant associations between n-3 PUFA and postpartum depression. In the first study, women with postpartum depression had slower normalization of functional DHA status after pregnancy, than their non-depressed counterpart.[47] In the second study, women with postpartum depression had lower DHA and total n-3 PUFA levels than their non-depressed counterpart.[48]

In sum, the reported significant inverse relationships between depression and total n-3 PUFA in a number of studies, have not been observed in our study populations. However, the observed inverse relationship between individual n-3 PUFA (i.e. ALA, EPA and DHA) and depression in this thesis, is consistent with findings of studies that have used different research designs and settings and study groups. This reinforces the evidence in favor of causality.

***5. Experimental evidence and reversibility***

Our findings of an inverse relationship between n-3 PUFA and depression are confirmed also by a number of randomized clinical trials. For example, an 8-week double-blind, placebo-controlled trial tested the effect of fish oil or placebo administration, on top of existing anti-depressive medication on 28 patients with major depression. The subjects received five capsules daily containing either fish oil (9.6 g/day) or placebo. Each fish oil capsule contained 440 mg EPA and 220 mg DHA. It was found that the fish oil group had a significantly lower score on the 21-item Hamilton Rating Scale for Depression than the placebo group.[33] However, it is not known whether the apparent therapeutic effect of fish oil was due to EPA, DHA or both. Moreover, it is not known whether fish oil has acted in synergy with and has potentiated the effect of antidepressant medication or has had independent anti-depressant properties of its own. Another 4-months double-blind, placebo-controlled trial tested the effect of fish oil (9.6 g/day) or placebo (olive oil) administration, on top of existing treatment on 30 patients with bipolar depression. It was

found that the fish oil group had a significantly longer period of remission than the placebo group.[49] Much like the previous study, it is not known whether the therapeutic effect of fish oil was due to DHA, EPA or both, nor is it known whether fish oil had antidepressant properties of its own or had acted in synergy with existing medication.

A double-blind, placebo-controlled trial tested the effect of different doses of EPA (1, 2, or 4 g/day) versus placebo over a 3-month period. Seventy patients with persistent depression received either placebo or EPA on top of background medication. It was found that EPA doses of 2 or 4 g/day did not cause a significant change in depression. However, 1 g of EPA/day caused a significant reduction on the Hamilton Depression Rating Scale score.[32] However, it is not known whether EPA exerted an independent anti-depressant effect or acted in synergy with background antidepressant medication. Furthermore, it is not known whether the apparent therapeutic effect of EPA was due to EPA alone, its longer-chain metabolite DHA, or both.

A different 4-week double-blind, placebo-controlled study, tested the efficacy of EPA versus placebo in treating recurrent unipolar depression. Patients were given 2 g EPA or placebo, in addition to existing anti-depressant therapy. EPA treatment resulted in significant reductions of depression. Patients receiving EPA had a mean reduction of 12.4 points on the Hamilton Depression Scale, while those receiving placebo had a 1.6 point reduction. The corresponding proportions of patients that achieved a 50% reduction in Hamilton depression score, were 60% and 10% respectively.[31] However, similar to the previous studies, it can not be ascertained whether EPA exerted an independent anti-depressant effect or has merely potentiated the antidepressant effect of existing medication. Moreover, it is not clear whether the therapeutic effect of EPA was due to EPA alone, its longer-chain metabolite DHA, or both.

Another double-blind, placebo-controlled pilot study tested the effect of ethyl-EPA and placebo in the treatment of borderline personality disorder. Thirty females received either 1 g EPA / daily or placebo over a 2-months period. EPA treatment resulted in significant decreases in depressive symptoms on Montgomery-Asberg Depression Rating Scale.[50] Again, it is not known whether the apparent therapeutic effect of EPA was due to EPA per se, its longer-chain derivative DHA, or both.

However, another randomized controlled clinical trail provided indications for an independent effect of EPA on depression.[51] The particular study examined the effect of fish

oil versus Antarctic Krill oil in premenstrual syndrome. Antarctic Krill oil has the same amount of DHA (120 mg/g) as fish oil. However, the former oil is very rich in phospholipids and has a higher EPA content than fish oil capsules. The EPA content of Antarctic Krill oil is 240 mg/g, while that of fish oil is 180 mg/g. Seventy women diagnosed with premenstrual syndrome received 2 g of fish oil or 2 g of Antarctic Krill oil daily, over a period of three months. Assessments at 45 and 90 days showed that women taking Antarctic Krill oil manifested significant improvements in the depressive symptoms of premenstrual syndrome, a fact not evidenced for those using fish oil. This indicates that the therapeutic effect of Antarctic Krill oil must have been due to the presence of phospholipids and/or the higher EPA content. It is noteworthy, that the women were not on anti-depressant medication. This precludes the possibility that the therapeutic effect of the n-3 PUFA in Antarctic Krill came as a result of synergy with existing anti-depressant medication.

However, it must be emphasized that not all randomized clinical trials of DHA or fish oil supplementation have yielded positive results. For example, randomized clinical controlled trials of DHA administration at doses of (2 g/day) and (200 mg/day) were not associated with significant reductions in depression.[52,52] Another randomized clinical controlled trial indicated that fish oil (8 g/day) administration did not lead to more favorable therapeutic outcomes than placebo (olive oil) administration in depressed patients.[54] It is of interest to note that whereas all three clinical controlled trials of EPA administration have produced significant positive results,[31,32,50] none of the two trials involving DHA administration have been associated with significant therapeutic outcomes.[52,53] On the other hand, three [33,49,51] of the four clinical controlled trials of fish oil (EPA and DHA) administration have been associated with positive therapeutic outcomes.[33,49,51,54] These observations appear to downplay the role of DHA as an independent predictor of depression. The extent to which DHA acts in synergy with EPA to exert an antidepressant effect is not known. Clearly more clinical controlled trials of DHA supplementation are needed.

A recent meta-analysis of 12 clinical trails investigating the effects of n-3 PUFA on depressed mood indicated that there is some evidence to support the therapeutic efficacy of n-3 fatty acids in reducing depression. However, the pronounced heterogeneity in results and in study populations and the short duration of the interventions prohibited firm conclusions on the effects of n-3 PUFA on depression. The authors suggested a need for

better designed larger clinical trials with sufficient statistical power for detecting clinically significant improvements.[55]

In sum, clinical controlled trials showed that EPA and DHA may reduce the risk of depression, but the current evidence is inconclusive. Clinical controlled trials of ALA administration in depressed patients have not yet been conducted. Also, cross-over clinical trials of n-3 PUFA administration have not as yet been carried out. Consequently, it is not known whether discontinuation of n-3 PUFA administration is accompanied by a corresponding increase in depression symptomatology or a deterioration of depression, a fact that would reinforce the existing evidence in favour of causality. In other words, on the basis of the existing evidence conclusions on the reversibility criterion of causality can not be drawn.

### *6. Biological plausibility*

#### *6.1 EPA and depression*

Similar to studies by other investigators, our study on adolescents also appears to provide indications for a possible independent effect of EPA on depression. Specifically, in spite of the fact that both adipose EPA and DHA had been included as predictors simultaneously in the regression analysis of our study, it was adipose EPA rather than DHA that attained significance. This indicates that the observed relationship between depression and EPA in our adolescents, was not mediated by DHA. However, it should always be born in mind, that the levels of the particular fatty acids (i.e. EPA, DHA) in the adipose tissue may not necessarily closely match those in the brain. It is not yet known the extent to which adipose tissue fatty acids reflect or correlate with brain fatty acids. Although the adipose tissue does supply the brain with fatty acids, it appears that it does so in a rather indirect fashion. Specifically, as a result of hydrolysis of adipose tissue triacylglycerols by adipose triglyceride lipase and hormone sensitive lipase, free fatty acids enter the circulation. Free fatty acids in turn, either in a non-esterified form or esterified in lysophosphatidylcholine supply cells, tissues and organs including the brain with fatty acids. There are indications that the brain preferentially incorporates esterified rather than non-esterified fatty acids. Several proteins are involved in the transfer of fatty acids to the brain such as very-long-chain acyl-coenzyme A synthetase, fatty acid binding protein and fatty acid transport protein.[56] Furthermore, it has been reported that the brain is capable of synthesizing DHA

from shorter-chain n-3 PUFA precursors.[57] The fact that the relationship of depression with adipose EPA in our adolescents is not mediated by adipose DHA does not necessarily mean that it is also not mediated by brain DHA. In light of the fact that DHA is a metabolite of EPA, the possibility that the decreasing adipose EPA with increasing depression in our adolescents is associated with or translates to decreases in brain DHA, can not be ruled out. It has been reported that fatty acids esterified to red blood cell membrane phospholipids closely match those of neuronal membranes. However, erythrocyte membrane phospholipids fatty acid measures have not been implemented in our studies. Had these measures been obtained, we would have had an indication about the levels of EPA as well as DHA in the brain. It is not possible, therefore, to discern whether EPA has been an independent predictor of depression in our adolescents.

Nevertheless, it is possible that EPA may exert an independent effect on depression via resolvin E1 (RvE1), a newly discovered oxygenase metabolite of EPA.[58] Specifically, it has been reported that depression is an inflammatory illness. On the other hand, RvE1 is implicated in the resolution phase of acute inflammation, hence the term resolvin. RvE1 has been reported to be a potent anti-inflammatory compound.[59] The extent to which RvE1 inhibits the activation of the inflammatory response system in depression is not known. Whether RvE1 is related to depression is not yet known. Studies that would shed light on this issue are therefore needed.

Depression is characterized by elevated pro-inflammatory cytokines such as IL-1, IL-2, IL-6 and TNF-alpha.[60-62] On the other hand, it has been reported that EPA and DHA suppress the synthesis of these pro-inflammatory cytokines by human lymphocytes and endothelial cells.[63,64] Also, dietary supplementation with EPA and DHA has been reported to suppress the production of IL-1, IL-2, IL-6 and TNF-α by human mononuclear cells.[65] The observed inverse relationship between EPA and depression in our adolescents, therefore, may stem from the inhibiting effect of EPA and/or that of its longer-chain metabolite DHA on the production of the particular cytokines.

Finally, some plausible explanation for the inverse relationship between EPA and depression, in our adolescents, may relate to a possible neuroprotection conferred by EPA. Specifically, depression has been characterized by neuronal atrophy and volume loss in the hippocampus. On the other hand, animal studies have shown that EPA had a neuroprotective effect on the hippocampus of rats exposed to gamma-irradiation [66,67] and lipopolysaccharide,[68] and reversed the age-related neurodegenerative changes in the rat

hippocampus, forebrain and cortex.[69-71] The neuroprotective effect of EPA appears to be mediated, among other things, by decreases in brain interleukin-1beta concentrations, reactive oxygen species production and vulnerability to beta-amyloid peptide, inhibition of p38 activation and apoptotic cell death, and inhibition of the consequences of c-Jun N-terminal kinase (JNK) activation.[66-71] A human case study of a treatment-resistant severely depressed and suicidal patient indicated that addition of EPA on top of background antidepressant treatment over a period of one month, led to a dramatic and sustained clinical improvement, and structural brain changes evidenced by cerebral magnetic resonance scanning.[72] Similar findings are indicated by another human case report. Specifically, introduction of EPA on top of existing antipsychotic medication in a treatment-resistant schizophrenic patient led to remission. 3D cerebral magnetic resonance spectroscopy scans indicated that the cerebral atrophy present the year prior to EPA administration was reversed after six months of EPA treatment.[73]

### *6.2 DHA and depression*

Much like EPA, the observed inverse relationship between DHA and depression in two of our adult studies, probably stems from the inhibiting effect of DHA on the production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6 and TNF-α. Another reason for the inverse relationship between adipose tissue DHA and depression, may involve dopaminergic and serotonergic pathways. DHA feeding has been reported to lead to increases in the serotonin and dopamine levels in the rat hippocampus. Dietary supplementation of DHA and arachidonic acid averted a decrease in dopaminergic and serotonergic neurotransmitters in animal frontal cortex. Moreover, n-3 PUFA deficiency has been linked with lower dopamine levels in rats. Cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) levels reflect central concentrations of serotonin and dopamine respectively. It has been reported that plasma DHA correlates positively with CFS 5-HIAA and HVA levels in healthy humans. Given that depression is characterized by reduced dopamine and serotonin levels, the observed inverse relationship between adipose tissue DHA and depression in our studies, may reflect a stimulatory effect of DHA on serotonin and dopamine synthesis. Finally, another explanation for the inverse relationship between adipose tissue DHA and depression, may stem from a possible neuroprotective effect of DHA on the hippocampus. Specifically, depression is characterized by neuronal atrophy and volume loss in the hippocampus. Insufficient dietary intake of n-3 PUFA has been reported to result in diminished nerve

growth factor levels in rat hippocampus. Deficiency in DHA intake has been reported to lead to decreases in neuron size in the hippocampus of rats. Moreover, DHA has been reported to protect rat hippocampal cultures from glutamate-induced cytotoxicity. DHA has been credited with neuroprotective and neurotrophic properties in a number of animal studies.

### *6.3 ALA and depression*

It must be emphasized that most of the studies that have demonstrated a significant association of depression with n-3 PUFA, have done so for ALA metabolites such as EPA and DHA rather than ALA per se.[74,75] Although the contention is that the association of depression with ALA should be mediated by its longer-chain derivatives such as EPA and DHA, there are indications that ALA per se may be an independent predictor of depression. Specifically, in spite of the fact that adipose EPA and DHA had been included as predictors together with ALA in the multivariate analysis in one of our studies, it was adipose ALA rather than EPA or DHA that attained significance. This indicates that the observed relationship between depression and ALA in our elderly group, was not mediated by EPA or DHA. Furthermore, it is unlikely that the relationship between depression and ALA in our subjects was due to reduced food and energy intake or differences in socioeconomic status. For example, in all likelihood, the significantly lower adipose ALA in our depressed sub-group of elderly subjects relative to that of the non-depressed one has not been reflecting socioeconomic differences or differences in food and caloric intake. Specifically, the fact that the two different sub-groups consisted of farmers, rules out the possibility that the lower adipose tissue ALA levels in our depressed sub-sample have been reflecting socioeconomic differences from the non-depressed one. Furthermore, given that two sub-groups did not differ in BMI or serum cholesteryl ester C18:2n-6, C16:0 and C20:4n-6, it is unlikely that the significant difference in adipose ALA has been due to differences in food and energy intakes in the two different sub-groups. These observations appear to advocate for an independent effect of ALA on depression in our elderly group.

However, it should always be born in mind, that the levels of the particular fatty acids (i.e. EPA, DHA) in the adipose tissue may not necessarily closely match those in the brain. The fact that the relationship of depression with adipose ALA in our elderly subjects is not mediated by adipose EPA or DHA does not necessarily mean that it is also not mediated by brain EPA or DHA. Given that EPA and DHA are metabolites of ALA, it is possible that the decreasing adipose ALA with increasing depression in our subjects is associated or

coupled with decreases in brain EPA and DHA levels. However, this can not be known since brain fatty acid measures were not implemented in our studies. To the best of our knowledge, studies relating adipose with brain fatty acids have not yet been conducted and are, therefore, needed. It has been reported that fatty acids esterified to red blood cell membrane phospholipids closely match those of neuronal membranes. However, erythrocyte membrane phospholipids fatty acid measures have not been implemented in our study. Had these measures been obtained, we would have had an indication about the levels of EPA as well as DHA in the brain. It is not possible, therefore, to conclude that adipose ALA has been an independent predictor of depression in our study.

Nevertheless, it is assumed that the relationship between ALA and depression must be mediated by its longer-chain metabolites EPA and/or DHA. This is supported by the fact that the brain is rich in arachidonic acid, EPA and DHA (particularly DHA), not ALA.[56] However, although the brain has some capacity for de novo synthesis of long-chain PUFA from their shorter-chain precursors, its fatty acid supply depends primarily on transport of preformed long-chain PUFA (e.g. DHA) via the blood-brain barrier.[56] Moreover, it has been reported that most of ALA is oxidized and used as metabolic fuel and that only a small proportion of it is metabolized to EPA and DHA.[76] Most studies have shown that in human adults the synthesis of EPA from ALA is restricted, while that of DHA from ALA is severely restricted.[76,77] It has been reported that the effects of consuming ALA-rich oils are less pronounced than those of EPA-rich oils and that dietary ALA is less effective than dietary DHA in increasing tissue and brain DHA levels.[78-80] Given the restrictions in converting ALA to EPA and DHA in humans and the reliance of the brain on preformed long-chain n-3 PUFA, it is reasonable to assume that brain EPA and DHA levels are more strongly related to adipose EPA and DHA than adipose ALA levels. Assuming that the relationship of depression with n-3 PUFA (including ALA) is mediated by brain EPA and DHA, it would be reasonable to expect that depression should correlate to adipose EPA and DHA rather than ALA. However, this has not been the case in our elderly subjects.

One possibility is that the capacity of converting ALA to EPA and DHA in our subjects was sufficient and not as restricted as that previously thought.[76,77] Another possibility is that the relationship of depression with adipose ALA in our subjects was not mediated by EPA or DHA but was due to some other mechanism. Depression is characterized by elevated cytokines such as IL-1, IL-2, IL-6 and TNF-alpha. On the other hand, human studies have reported decreases in IL-1, IL-6 and TNF-alpha synthesis in response to dietary

supplementation with ALA.[81,82] However, it is not clear whether these decreases in IL-1, IL-6 and TNF-alpha levels have been due to ALA or its longer-chain metabolites EPA and DHA. Indeed, not only ALA, but perhaps even more so EPA and DHA are also capable of reducing the synthesis of pro-inflammatory cytokines. Specifically, in vitro studies have shown that EPA and DHA suppress IL-6 synthesis by human endothelial cells, and the production of IL-1, IL-2, IL-6, TNF-α and INF-γ by human lymphocytes.[63,64] Also, dietary supplementation with EPA and DHA has been reported to suppress the production of IL-1, IL-2, IL-6 and TNF-α by human mononuclear cells.[65] Given that cytokines such as IL-1, IL-2, IL-6 and TNF-α have been reported to relate positively to depression, the observed inverse relationship between ALA and depression, in one of our studies and those of others, may stem from the inhibiting effect of ALA or its metabolites EPA and DHA on the production of the particular cytokines.

It is, however, possible that adipose tissue ALA may relate to depression via decreasing pro-inflammatory cytokine production independently of its longer-chain metabolites EPA and DHA. Specifically, unlikely previously thought, the adipose tissue is not merely a depot of metabolic fuel but an endocrine organ implicated in immune functions. The adipose tissue is a major production site for a number of hormones, anti-inflammatory as well as pro-inflammatory cytokines, including IL-6, IL-1 and TNF- α.[83,84] We propose a novel mechanism that could account for the inverse relationship between depression and adipose tissue ALA in our subjects. Specifically, adipose tissue ALA may inhibit the production of pro-inflammatory cytokines (i.e. IL-6, IL-1 and TNF-α) by the adipose tissue rather than some different medium (e.g. monocytes). Studies that would examine this possibility, namely that adipose ALA per se, rather than its longer-chain metabolites EPA and DHA, has an inhibiting effect on the production of IL-6, IL-1 and TNF-α by the adipose tissue are therefore needed.

Whatever the case may be, adipose ALA does not derive from fish or fish oil intake. The inverse relationship between adipose ALA and depression in our elderly subjects, therefore, suggests that long-term n-3 PUFA intake from dietary sources other than fish and/or fish oil (e.g. plant food) may also be related to depression.

In sum, there is biological evidence in support of the observed inverse relationship between depression and adipose tissue ALA, EPA and DHA in our adolescent, adult and elderly groups. This strengthens the existing evidence in favor of a causal association between n-3 PUFA and depression. ALA, and possibly to a greater degree its metabolites EPA and

DHA, have been reported to inhibit the formation of cytokines such as IL-1, IL-2, IL-6 and TNF-α. Given that depression is associated positively with these cytokines, the observed inverse relationship between ALA and depression, probably derives from the inhibiting effect of ALA or its metabolites EPA and DHA on the production of the particular cytokines. It is possible, that adipose ALA may have an independent effect on depression via decreasing the production of pro-inflammatory cytokines by the adipose tissue. Similar to ALA, the observed inverse relationship between EPA and depression, probably derives from the inhibiting effect of EPA and/or its metabolite DHA on the production of IL-1, IL-2, IL-6 and TNF-α. It is possible that adipose EPA may have an independent effect on depression by reducing inflammation through its metabolite ReV1 or via a possible neuroprotective and/or neurotrophic effect on the hippocampus. The inverse relationship of DHA with depression may stem from the inhibiting effect of DHA on pro-inflammatory cytokine production, a stimulatory effect of DHA on serotonin and dopamine synthesis and possible neuroprotection on the hippocampus.

## CONCLUSIONS

This thesis focused on the relationship between adipose n-3 PUFA and degree of depressive symptomatology in predominantly non-depressed adolescent, adult and elderly subjects. In all age groups studied, there were significant inverse relationships between degree of depression and different n-3 PUFA. ALA emerged as a significant predictor of depression in one study (elderly). EPA was a significant predictor of depression in one study (adolescents). DHA was found to be significantly related to depression in two studies involving adults. The studies of the present thesis are the first literature reports of an association between depression and a biomarker of long-term n-3 PUFA intake in humans. The results of these studies indicate that a low long-term dietary intake of different n-3 PUFA is associated with an increased risk for depression in adolescents, adults and the elderly. Our findings are supported by cross-cultural, cross-sectional, case-control and prospective cohort studies. The results of the studies of this thesis and those of other studies are in favor for a causal role of n-3 PUFA in depression. However the criterion of reversibility was not fulfilled. All other criteria are suggestive for a causal relationship between n-3 PUFA and depression, namely temporality, strength of association, dose-response relationship, consistency of association, experimental evidence and biological plausibility. However, results of cross-over clinical trials on n-3 PUFA and depression are

needed before definite conclusions concerning an etiological role of n-3 PUFA in depression can be drawn.

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# SUMMARY

In this thesis, the results of cross-sectional studies on the relationship of depression with adipose tissue polyunsaturated fatty acids of the n-3 series have been described. Depression, the most common psychiatric disorder in adults, is characterized by increased morbidity and mortality. The incidence of depression has increased during the last 100 years. It is expected that depression will become one of the leading causes of disability worldwide by the year 2030. There are indications that depression is significantly related to short-term n-3 PUFA intake. A number of studies that have used objective indices or biomarkers of short-term fatty acid intake have indicated significant inverse relationships between depression and short-term n-3 PUFA intake. Up until now, however, no studies have investigated whether depression relates also to biomarkers of long-term or habitual n-3 PUFA intake.

The aim of this thesis is to investigate whether adipose tissue n-3 fatty acids, an objective index or biomarker of long-term or habitual n-3 PUFA intake relates to depression. The study populations in this thesis were comprised of generally healthy adolescent, adult and elderly volunteers from the island of Crete. With the exception of the elderly group that consisted solely of males, all other age-groups consisted of both male and female participants.

Anthropometric measures were taken and data concerning subjects' smoking habits and education were collected. Long-term or habitual n-3 fatty acid intake was assessed through the use of the adipose tissue as a biomarker. The fatty acid composition of adipose tissue has been reported to reflect dietary intake of the preceding 2 to 3 year period. Adipose samples were aspirated from the gluteal adipose tissue and n-3 PUFA were determined by gas chromatography. Depressive symptomatology was assessed through the use of well-known, standardized, widely used self-rating depression scales such as the Beck Depression Inventory (BDI), the Zung Self Rating Depression Scale (ZSRDS), the Center for Epidemiologic Studies Depression Scale (CES-D) and the 15-item version of the Geriatric Depression Scale (GDS-15).

Significant relationships between different n-3 PUFA and depression were not manifested in our adolescent group (chapter 2). This group consisted of 90 healthy adolescents from an urban community of Crete. There were 54 girls and 36 boys aged 13 to 18. The average age was 15.2 years. In the same adolescent group, inclusion of serum adiponectin along with other covariates in the statistical analysis, yielded a significant inverse relationship (Beta=-0.23, t=-2.09, $p<0.05$) between scores on the Beck Depression Inventory (BDI) and

adipose tissue EPA (chapter 3). This inverse relationship between depression and adipose EPA in our adolescent group is the first literature report of a relationship between depression and an individual n-3 fatty acid in adolescents. Furthermore, this is the first literature report of a significant relationship between depression and a biomarker of long-term EPA intake in humans.

A significant inverse correlation between depression and adipose tissue DHA was observed in one of our adult groups. The particular group consisted of 247 healthy lawyers from an urban community of Crete. There were 146 males and 101 females, aged 24 to 69. The mean age was 39 years. A comparison of mildly depressed adults and non-depressed ones indicated significantly lower (-34.6%, $p<0.05$) adipose tissue DHA levels in the former sub-group. The observed relationship could not be attributed to age, gender, body mass index, or educational level (chapter 4). The inverse relationship between depression and adipose DHA in this adult group is the first literature report of a relationship between depression and a biomarker of long-term DHA intake in humans. The inverse relationship between depression and adipose tissue DHA was also observed in a second adult group. This group consisted of 130 healthy adults (59 males, 71 females) aged 22 to 58. The mean age was 36.9 years. The observed relationship (Beta=-0.22, t=-2.7, $p<0.008$) was not due to age, gender, body mass index, smoking or educational level (chapter 5). In third adult group, depression was significantly related to adipose tissue ALA levels ($r=-0.17$, $p<0.05$). This association was not statistically significant when the confounding effect of age, gender, body mass index and education was taken into account (chapter 6). This group, a representative sample of a rural community of Crete, comprised of 394 healthy subjects (175 males, 219 females) aged 18 to 60. The average age was 44.7 years.

An inverse relationship between depression and adipose tissue ALA levels was observed in a group of elderly. The particular group, the surviving sample of the Greek Seven Countries Study group, consisted of 150 healthy males aged 80 to 96. The average age was 84 years. Depression correlated negatively with adipose tissue ALA levels (Beta=-0.30, t=-2.4, $p<0.02$) in these elderly subjects. Depressed males had significantly lowered (-10.5%, $p<0.02$) adipose ALA levels than non-depressed ones (chapter 7). The inverse relationship between depression and adipose tissue ALA levels in our elderly group constitutes the first literature report of a relationship between a biomarker of long-term ALA intake and depression in the elderly (chapter 7).

Finally, in the same elderly group, inclusion of serum cholesteryl ester fatty acids, an indicator of the short-term intake of n-3 PUFA, in the statistical analysis did not change the results obtained. Namely, the observed significant relationship between depression and adipose ALA levels was independent of the serum cholesteryl ester fatty acids (chapter 8).

The results of this thesis demonstrate that in the different age-groups studied, there were significant inverse relationships between degree of depression and different adipose tissue n-3 PUFA. Adipose tissue EPA was significantly inversely associated with depression in adolescents. Similar relations were found for DHA in adults and for ALA in the elderly. This indicates that a high long-term intake in particular n-3 fatty acids is associated with a lower risk of depression in different age-groups. However, an all-embracing explanation for the relations observed for the different n-3 PUFA in the different age-groups is not yet available.

Our findings are supported by cross-cultural, cross-sectional, case-control and prospective cohort studies. The results of the studies reported in this thesis and those of other studies support a causal role of n-3 PUFA in depression. There is some evidence from small clinical trials that EPA and DHA lower the risk of depression. However, the results of these trials are very heterogeneous. With the exception of the criterion of reversibility, all other criteria are suggestive for a causal relationship between n-3 PUFA and depression, namely temporality, strength of association, dose-response relationship, consistency of association, experimental evidence and biological plausibility. However, results of high quality cross-over clinical trials on the individual n-3 PUFA and depression are needed before definite conclusions concerning an etiological role of n-3 PUFA in depression can be drawn (chapter 9).

# SAMENVATTING

In dit proefschrift zijn de resultaten beschreven van transversale onderzoeken naar de verbanden tussen depressie en n-3 meervoudig onverzadigde vetzuren. Depressie, de meest voorkomende psychiatrische stoornis bij volwassenen, wordt gekarakteriseerd door een verhoogde ziekte- en sterftelast. De incidentie van depressie is de afgelopen 100 jaar toegenomen. Er wordt verwacht dat depressie wereldwijd een van de belangrijkste oorzaken van functionele beperkingen zal zijn in 2030. Er zijn aanwijzingen dat depressie significant samenhangt met de korte termijn inname van n-3 vetzuren. Een aantal onderzoeken waarin objectieve indices of biomerkers van de korte termijn inname van vetzuren zijn gebruikt, laten een significant invers verband zien tussen depressie en de korte termijn inname van n-3 vetzuren. Tot nu toe is echter niet onderzocht of depressie ook met biomerkers van de lange termijn inname van n-3 vetzuren samenhangt.

Het doel van dit proefschrift is te onderzoeken of n-3 vetzuren in vetweefsel, een objectieve index of een biomerker van de lange termijn inname van n-3 vetzuren, gerelateerd zijn aan depressie. De onderzoekspopulaties die in dit proefschrift zijn beschreven bestaan uit over het algemeen gezonde adolescenten, volwassenen en ouderen op het eiland Kreta. Met uitzondering van de groep ouderen die geheel uit mannen bestond, bevatten de overige groepen zowel mannen als vrouwen.

Er zijn anthropometrische maten gemeten en gegevens verzameld over rookgewoonten en opleiding. Als indicator van de lange termijn of de gebruikelijke inname zijn vetzuren in vetweefsel bepaald. Vetweefsel weerspiegelt de inname van vetzuren over de afgelopen 2 tot 3 jaar. Vetzuren werden gas-chromatografisch bepaald in een kleine hoeveelheid vet afkomstig uit de bil. Depressieve symptomen werden vastgesteld aan de hand van bekende gestandaardiseerde en veelvuldig gebruikte zelf-ingevulde vragenlijsten zoals de Beck Depression Inventory (BDI), de Zung Self Rating Depression Scale (ZSRDS), de Center for Epidemiologic Studies Depression Scale (CES-D) en de 15-item versie van de Geriatric Depression Scale (GDS-15).

Bij adolescenten werden geen significante verbanden gevonden tussen verschillende n-3 vetzuren en depressie (hoofdstuk 2). Deze groep bestond uit 90 gezonde adolescenten afkomstig uit een stedelijke gemeenschap op Kreta. Er namen 54 meisjes en 36 jongens van 13-18 jaar aan dit onderzoek deel. De gemiddelde leeftijd was 15,2 jaar. In dezelfde groep adolescenten werd na inclusie van serum adiponectine en andere covariaten in het

statistische model een significant invers verband (Beta=-0,23, t=-2,09, p<0,05) vastgesteld tussen de BDI scores voor depressie en het n-3 vetzuur eicosapentaeenzuur (EPA) in vetweefsel (hoofdstuk 3). Dit is het eerste artikel waarin een relatie is beschreven tussen depressie en een individueel n-3 vetzuur bij adolescenten. Bovendien is het de eerste keer dat een significante relatie tussen depressie en een biomerker van de lange termijn inname van EPA is vastgesteld bij mensen.

Bij een groep volwassenen werd een inverse correlatie gevonden tussen depressie en het n-3 vetzuur docosahexaeenzuur (DHA) in vetweefsel. Deze groep bestond uit 247 (146 mannen en 101 vrouwen) gezonde advocaten in een stedelijk gebied op Kreta. De gemiddelde leeftijd was 39 jaar. Volwassenen met milde depressie hadden significant lagere (34,6%, p<0.005) DHA niveau's in vetweefsel dan mensen zonder depressie. Deze relatie kon niet worden toegeschreven aan leeftijd, geslacht, body mass index of opleiding (hoofdstuk 4). Dit is het eerste artikel waarin een inverse relatie is beschreven tussen depressie en een biomerker van de lange termijn inname van DHA bij volwassenen. Bij een tweede groep volwassenen werd eveneens een inverse relatie gevonden tussen depressie en DHA in vetweefsel. Deze groep bestond uit 130 gezonde volwassenen (59 mannen, 71 vrouwen) van 22-58 jaar. De gemiddelde leeftijd was 36,9 jaar. De waargenomen associatie (Beta=-0,23, t=-2,7, p<0,008) kon niet worden toegeschreven aan leeftijd, geslacht, body mass index, roken of opleidingsniveau (hoofdstuk 5). In een derde groep volwassenen was depressie significant invers gecorreleerd met het n-3 vetzuur ALA in vetweefsel (r=-0,17, p<0,05). Deze relatie was niet statistisch significant als het verstorende effect van leeftijd, geslacht, body mass index en opleiding in ogenschouw werd genomen (hoofdstuk 6). Dit was een representatieve steekproef van een plattelandsgemeenschap op Kreta die bestond uit 394 gezonden personen (175 mannen. 219 vrouwen) van 18-60 jaar. In dit onderzoek werd ook geen relatie gevonden tussen depressie en n-3 vetzuren in serum fosfolipiden.

Bij ouderen werd een relatie tussen depressie en ALA in vetweefsel vastgesteld. Deze groep bestond uit 150 mannen van 80-96 jaar die deel uit maken van het Kretenzer cohort van de Zeven Landen Studie. De gemiddelde leeftijd was 84 jaar. Depressie was invers gecorreleerd met ALA in vetweefsel (Beta=-0,30, t=-2,4, p<0,02) bij deze ouderen. Mannen met depressie hadden significant lagere ALA niveau's dan mannen zonder depressie (hoofdstuk 7). Dit is het eerste artikel waarin de inverse relatie tussen depressie en ALA in vetweefsel bij ouderen is beschreven.

Ten slotte werd in dezelfde groep ouderen aangetoond dat inclusie van serum cholesterolesters, een indicator van de korte termijn inname van n-3 vetzuren, in het statistische model, de verkregen resultaten niet veranderde. Namelijk, de waargenomen significante relatie tussen depressie en ALA in vetweefsel was onafhankelijk van vetzuren in serum cholesterolesters (hoofdstuk 8)

De resultaten in dit proefschrift laten zien dat in de verschillende bestudeerde leeftijdsgroepen, er significante inverse relaties waren tussen de mate van depressie en verschillende n-3 vetzuren in vetweefsel. EPA in vetweefsel was significant invers geassocieerd met depressie bij adolescenten. Soortgelijke relaties werden gevonden voor DHA bij volwassenen en ALA bij ouderen. Dit laat zien dat een hoge inname van verschillende n-3 vetzuren gedurende een lange periode is geassocieerd met een lager risico op depressie in verschillende leeftijdsgroepen. Echter een alles omvattende verklaring voor de waargenomen relaties voor de verschillende n-3 vetzuren in de verschillende leeftijdsgroepen is nog niet beschikbaar.

Onze bevindingen worden gesteund door de resultaten van cross-cultureel-, transversaal-, patiënt-controle- en prospectieve cohort onderzoeken. De resultaten van de onderzoeken die gerapporteerd zijn in dit proefschrift en die van andere onderzoeken ondersteunen een causale rol van n-3 vetzuren in relatie tot depressie. Er is ook enig bewijs dat in kleine klinische trials EPA en DHA het risico van depressie verlagen. Echter de resultaten van deze trials zijn erg heterogeen. Met uitzondering van het criterium omkeerbaarheid, zijn alle andere criteria suggestief voor een causale relatie tussen n-3 vetzuren en depressie, namelijk, tijdsvolgorde, sterkte van de associatie, dosis-response relatie, consistentie van de associatie, experimenteel bewijs en biologische plausabiliteit. Echter, resultaten van cross-over trials naar de relaties tussen individuele n-3 vetzuren en depressie zijn nodig voordat definitieve conclusies over de etiologische rol van n-3 vetzuren bij het ontstaan van depressie getrokken kunnen worden (hoofdstuk 9).

# ACKNOWLEDGEMENTS

First of all, I would want to thank Professor Anthony Kafatos for giving me (not just me, as a matter of fact) a job and opportunity. It is quite likely, had I not been offered this employment opportunity, I might have been employed at some different field alien to my interests (God knows what). I would want to thank Professor Kafatos for his passion in research (in my opinion, something not too common among Greek Professors) and for his endless efforts to raise funds and organize and direct the different studies of this thesis. I would want to express my deepest gratitude to him, for his trust in me and my ability, and for his literally unlimited support and assistance throughout this endeavour. Also, I would want to thank him for his inspiration and invaluable advice, and the countless hours spent organizing and discussing about this thesis. I need to most emphatically stress the invaluable contribution of Professor Daan Kromhout in this project. I want to thank him for his support, inspiration, and constant feedback and advice, and the literally endless hours spent reviewing, modifying and proofreading this thesis. I would want to thank Professor Kromhout also for helping me the most with paperwork required by the graduate school as well as the editing and printing of this thesis. I would want to thank the four laboratories that carried out the different biochemical analyses with all their staff. Indeed, the work done by these four labs was the backbone of this investigation. Specifically, I would want to thank our Laboratory at the Department of Social and Preventive Medicine and Nutrition at the University of Crete, the Laboratory of Toxicology of the National Institute of Public Health and the Environment in Bilthoven, the Netherlands, the Laboratory of Food Chemistry, Biochemistry and Physical Chemistry at the Department of Science, Dietetics and Nutrition, Harokopio University, Greece, and the Laboratory of Endocrinology Diabetes and Metabolism, Department of Internal Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA. I would want to thank a number of good colleagues and co-authors, including Michael Kiriakakis, George Tsibinos, Nick Kalogeropoulos, Nick Andrikopoulos, Eugene Jansen, Hans Cremers, Carlo Strien, Joanna Moschandreas, Manolis Linardakis, Professor Christos Mantzoros, Sofia Flouri and my friend Christos Hatzis, without the assistance of which this project would not have been possible. I would like to thank the members of my Thesis Review Committee Professors E.G. Schouten, F.G. Zitman, G. Hornstra and P. Rogers, for their precious time spent reviewing this thesis. I would like to acknowledge the contribution of Mrs Lous Duym in

the editing and printing of this thesis. I would like to thank Joseph R. Hibbeln, M.D. for his interest, feedback and inspiration and the articles sent. Special thanks to Catherine Brusten, PhD graduate, Oxford Brookes University for her support. And finally, last but not least, I would want to thank my family for their continued and unconditional support.

# ABOUT THE AUTHOR

George Mamalakis was born in Rethymnon, Crete, Greece, in 1958. He attended Deree College, Athens, Greece, between 1976 and 1981, and he obtained a Bachelor of Arts (BA) in Psychology. He attended Austin Peay State University, TN 37044, U.S.A., between 1981-1982 and between 1985-1986. He obtained a Master of Arts (MA) in Psychology, with a Grade point average (GPA): (3.793 on a 0-to-4 scale). His Master thesis was on Interpersonal attraction and defensive style. It was published in the Journal of Social Psychology, 1988, 128(2), 275-277. In 1988-1989, he worked for a provincial cultural/educational organization (NELE), as a lecturer. He presented applied psychology topics to target audiences such as youth, elderly and groups of parents. Personal and/or family problems were dealt with on a group basis. He has been a researcher at the Department of Social and Preventive Medicine, Medical School, University of Crete, Iraklion, Crete, Greece, since 1989. The research themes worked on by the Author at the Department of Social and Preventive Medicine include surveys on the health status and disease risk factors (i.e. obesity indices, arterial pressure, serum chemistries, physical activity and physical fitness, dietary habits, adipose tissue fatty acid profiles, smoking and alcohol consumption) in different populations and age-groups in Crete, cross-sectional studies on the relationship between psychological constructs such as Type-A behavior, anxiety and depression, and essential fatty acids, a case-control study of prostate phospholipid and adipose tissue fatty acid profiles in prostate cancer v/s hyperplasia patients, prospective studies on the heart disease risk-factor status in the Cretan population of the Seven Countries Study over a 30-year period, and on the effects of health education interventions on physical activity, aerobic capacity and fitness indices and obesity in children, and, a cross-over study of the effects of a monounsaturated fat enriched diet on serum lipids in subjects with familial history of Coronary Artery Disease. From 2002 till the present date, the Author has been working on the topic of n-3 polyunsaturated fatty acids in the adipose tissue and depression, described in this thesis. For a more detailed account of the research engaged in by the Author, please refer to the List of Publications.

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Printing: Ponsen & Looyen BV, Wageningen, The Netherlands