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# Serum dihomo-gamma-linolenic acid level is inversely associated with the risk of depression. A 21-year follow-up study in general population men

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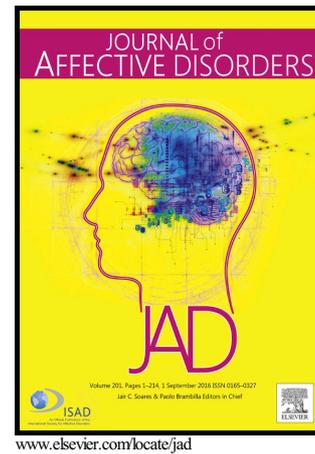
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## Author's Accepted Manuscript

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**Serum dihomo- $\gamma$ -linolenic acid level is inversely associated with the risk of depression. A 21-year follow-up study in general population men.**

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## **Abstract**

## **Background**

Depression is a major public health challenge worldwide, and polyunsaturated fatty acids (PUFAs), especially n-3 PUFAs, have been found to inversely associate with the risk of depression. However, only few cross-sectional studies have investigated the association between dihomo- $\gamma$ -linolenic acid (DGLA), an n-6 PUFA with anti-inflammatory effects, and depression. The aims of the present study were to examine an association between serum DGLA and the risk of depression, and to study whether the potential association is mediated via inflammation.

## Methods

A 20-year prospective Kuopio Ischaemic Heart Disease Risk Factor (KIHD) follow-up study was conducted from 1984 to 1989 with 2,179 middle-aged and older Finnish men (42 to 60 years old at baseline). The baseline concentrations of serum fatty acids, including DGLA, were determined. A hospital discharge diagnosis of depression was used as the main outcome and obtained from linkage to National Hospital Discharge Register. Serum C-reactive protein (CRP) levels were measured to assess inflammation.

## Results

An inverse association between serum DGLA concentration and incidence of depression was found after adjustment for several potential confounders (Hazard ratio HR 0.53, CI 0.36-0.79,  $P=0.002$ ). The association between DGLA and depression was not dependent on inflammation ( $P$ -interaction=0.618).

## Limitations

Our findings may not be generalizable to individuals below middle-age or women. Moreover, we were unable to consider cases with mild depression in the longitudinal setting.

## Conclusions

Higher serum DGLA concentrations may predict lower risk of develop depression in elderly men. Further studies are warranted to address potential mechanisms as mechanism behind this association remains unclear.

## Abbreviations

(AA), Arachidonic acid; (BMI), Body mass index; (CVD), Cardiovascular disease; (CRP), C-reactive protein; (DGLA), Dihomo- $\gamma$ -linolenic acid; (DHA), Docosahexaenoic acid; (EPA), Eicosapentaenoic acid; (GABA), Gamma-aminobutyric acid; (GLA), Gamma-linolenic acid; (HR), Hazard ratio; (HPL), Human Population Laboratory; (ICD), International Classification of Diseases; (KIHD) study, Kuopio Ischaemic Heart Disease Risk Factor; (LTPA), Leisure-Time Physical Activity; (LA), Linoleic acid; (PUFA), Polyunsaturated fatty acid; (PGE1), Prostaglandin E1; RQ, Rose Angina Questionnaire

**KEYWORDS:** Depression; Dihomo- $\gamma$ -linolenic acid; DGLA; Fatty acids; Prospective study

## 1. Introduction

Depression is the most common among psychiatric disorders and a risk factor of somatic diseases, such as acute myocardial infarction and cancer (Currier & Nemeroff, 2014). Risk factors of depression have been categorized in non-modifiable, modifiable, and contextual risk factors. Nutrition is considered as a modifiable risk factor for depression. Four polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), and dihomo-  $\gamma$  linolenic acid (DGLA) constitute more than 90% of lipids at brain synapses (Walsh, 2012). These fatty acids are needed for the higher functions of the brain and central

nervous system performance. DGLA is a 20-carbon n-6 (omega-6) PUFA, which is not an essential fatty acid, but can be endogenously synthesized in the body from linolenic acid (LA), an essential fatty acid, in humans. However, some factors, such as aging, can lead to a deficiency of this fatty acid (Wang et al., 2012).

Relationship between PUFAs and depression has been investigated among several populations in the world (Bloch & Hannestad, 2012). In many (Grosso et al., 2014; Kiecolt-Glaser et al., 2007), but not all studies (Hakkarainen et al., 2014; Ruusunen et al., 2011), high levels of both dietary and serum n-3 PUFAs, including EPA and DHA, have been associated with decreased prevalence or incidence of depressive symptoms or depression. The mechanism underlying the relationship between depression and PUFAs is unknown, but it is usually, at least partly, explained by the inflammation hypothesis of depression. Inflammation has been suggested to play a role in the development of depression (Kim et al., 2016), and a link between n-3 fatty acids and inflammatory markers in depressed subjects has been suggested (Rapaport et al., 2015). It is believed that metabolites of n-3 PUFAs, such as eicosanoids, may have more anti-inflammatory properties compared to the metabolites of n-6 PUFAs (Vanhala et al., 2012). However, eicosanoids derived from n-6 fatty acids have been shown to have potential anti-inflammatory actions as well (Calder, 2009). DGLA is an endogenous n-6 PUFA which can be further converted to prostaglandin E1 (PGE1), which has anti-inflammatory properties (Wang et al., 2012).

Few cross-sectional studies have found a positive association between concentrations of DGLA and depressive symptoms (Evans et al., 2012; Lotrich et al., 2013; Mamalakis et al., 2006) and one

cross-sectional study found an inverse association (Chalut-Carpentier et al., 2015). Due to limited data, the role of DGLA in the incidence of depression remains unclear, and there are no published prospective studies on the association. Therefore, the aim of this study was to examine an association between serum concentrations of DGLA and the risk of depression in middle-aged and older Finnish men. Secondly, we aimed to study whether the potential association between DGLA and depression is mediated via inflammation.

## **2. Methods**

### **2.1. Study population**

The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study was originally designed to explore cardiovascular disease (CVD) risk factors, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from Eastern Finland (Salonen, 1988). The baseline of study was carried out during 1984 to 1989. A total of 2,682 men who were 42-60 years old at baseline were recruited in two cohorts. The first cohort, enrolled from 1984 to 1986, consisted of 1,166 men aged 54 years old, and the second cohort included 1,516 men aged 42-60 years old, enrolled from 1986 to 1989. The baseline examinations were monitored by the 4-year examination round (1991–1993) in which 1,038 men from the second cohort (88% of those eligible) participated. All men from the second cohort were invited at the 11-year examination round (1998–2001), 854 men (95% of the eligible) participated. All eligible participants from the first and the second cohorts were invited to the 20-year examination round. The baseline characteristics of the entire study population have been described elsewhere (Salonen, 1988). A total of 2,101 participants were included and analyzed for in the prospective setting. The KIHD

study protocol was approved by the Research Ethics Committee of the University of Kuopio. Informed written consent was obtained from all participants before enrolment in the study.

## **2.2. Assessment of depressive symptoms and depression**

The 18-item Human Population Laboratory (HPL) Depression Scale was used to measure depressive symptoms at baseline, this scale is designed for screening of depressive symptoms among general population samples (Kaplan et al., 1987). A cut-off score of five or more was used (Tolmunen et al., 2004) to identify people with elevated depressive symptoms (n=262), and these subjects were excluded from the prospective analyses, leaving total 2,101 subjects for the final analyses.

Depressive disorders during the follow-up were obtained by computer linkage to the National Hospital Discharge Register in 2010. The average follow-up time for the cohort was 21.5 years. The International Classification of Diseases 8 (ICD-8) (years 1985–1986), ICD-9 (1987–1995) and ICD-10 (1996–2010) were used to diagnose depression. The following diagnoses were considered as depression: major depression (ICD-9: 2961-, ICD-10: F32.1-3, F33.1-3), a depressive disorder, otherwise unspecified (ICD-9: 2968A, ICD-10: F32.9, F33.9), chronic depression (ICD-8: 300.41, ICD-9: 3004A, ICD-10: F34.1) and adjustment disorder with depressive symptoms (ICD-9: 3090A).

## **2.3. Measurement of serum fatty acids**

Serum fatty acids were determined in one gas chromatographic run without pre-separation as described previously (Laaksonen et al., 2002). Serum fatty acids were extracted with chloroform-methanol. Chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was carried out with reference standards

purchased from NU-Check Prep Inc. (MA, USA). Each analyte had individual reference standard, and an internal standard was eicosane. Fatty acids were chromatographed in an NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, PA, since 1999 Agilent Technologies Inc.) with a flame ionization detector. Results were obtained in micromoles per liter. The coefficient of variation was 9.6% for LA, 11.7% for gamma-linolenic acid (GLA), 8.3% for DGLA and 9.2% for AA.

#### **2.4. Assessment of other variables**

The background information, including marital status and education, of study subjects was obtained from questionnaires as previously described (Salonen et al., 1992). Smoking status (never, past or current smoking), the type (cigarettes, cigars) and the amount smoked per day were evaluated using questionnaires. Alcohol consumption (grams/week) was calculated with a structured quantity-frequency method using the Nordic Alcohol Consumption Inventory for drinking behavior over the previous 12 months (Kauhanen et al., 1992). Dietary intake of nutrients and total energy intake were calculated based on 4-day food records (Voutilainen et al., 2001). The 12-Month Leisure-Time Physical Activity (LTPA) Questionnaire was used to assess leisure-time physical activity (Lakka & Salonen, 1992), and is described in more detail elsewhere (Laukkanen et al., 2009). The questionnaire was checked with an interview by a trained nurse and the energy expenditure from LTPA was expressed as kcal per day. The weight and height of the participants were measured by the study nurse, and the body mass index (BMI) was calculated as the ratio of weight in kilograms to the square of height in meters. A positive CVD history was coded based on the following criteria: first, all subjects had at least one of the following physician-diagnosed conditions: myocardial infarction, angina pectoris, other coronary conditions, cardiomyopathy,

cardiac insufficiency or stroke. Second, all also used nitrates at least once per week, and had angina pectoris according to the World Health Organization angina pectoris questionnaire (the Rose Angina Questionnaire, RQ), a validated instrument to assess symptoms of typical angina pectoris in the general population (Rose, 1962). Diabetes was defined as a self-reported physician-set diagnosis of diabetes. Serum C-reactive protein (CRP) was measured with an immunometric assay (Immulite High Sensitivity CRP Assay, DPC, Los Angeles, Calif., USA).

### **2.5. Statistical analysis**

The associations between baseline characteristics of the study subjects, including fatty acids, age, smoking status, marital status, education, alcohol intake, leisure-time physical activity, BMI, CVD history and prevalent diabetes, according to the hospital discharge diagnosis of depression were examined using independent sample *t*-test for continuous variables and chi-square test for categorical variables. In this model, depression was used as the dependent variable and baseline variables were used as independent variables. The association between serum DGLA concentrations at baseline and incidence of depression during the follow-up was examined by Cox regression hazard's model, adjusted for all the listed baseline variables. Standardized mean of DGLA was entered into the models. The moderation was examined with interaction between DGLA and CRP. All analyses were conducted with the SPSS statistical software (version 22; SPSS Inc., Chicago, IL). Two-tailed *P* values below 0.05 were considered to indicate statistical significance.

### **3. Results**

Fifty-eight men received a hospital discharge diagnosis of depression during the follow-up. Table 1 presents the baseline characteristics of the study participants according to a hospital discharge diagnosis of depression. Total energy intake and serum concentrations of DGLA at baseline were significantly associated with a hospital discharge diagnosis of depression during the follow-up.

Table 2 shows the prospective association between serum concentration of DGLA and incident of depression in three different statistical models. In all of these, there was a statistically significant inverse association between DGLA and the depression risk. The first model was adjusted for age and examination year (Hazard ratio HR 0.69, CI 0.51-0.94, P=0.018). Model 2 was adjusted for Model 1 and smoking status, marital status, alcohol consumption, BMI, history of CVD, history of diabetes and history of mental illness (Model 2; HR 0.65, CI 0.48-0.89, P=0.008). Finally, Model 3 was adjusted for Model 2 and serum concentrations of fatty acids including linolenic acid, EPA, DHA, ALA, GLA and AA (Model 3; HR 0.53, CI 0.36-0.79, P=0.002). Intake of fruits, berries, vegetables and red meat and nutrient intakes, including magnesium, zinc, vitamin B<sub>3</sub>, folate, cobalamin, fiber and vitamin C and E did not change the association between DGLA and depression (data not shown).

We also examined whether the association between DGLA and depression is dependent on inflammation. Correlation between DGLA and CRP at baseline was calculated (Correlation Coefficient=0.087, P<0.001). We did not observe a significant association between CRP and the incidence of depression nor a significant interaction between DGLA and CRP (P-interaction=0.618). Furthermore, adjusting the Model 3 with CRP did not change the results.

#### 4. Discussion

Our findings demonstrate that serum DGLA concentration is inversely associated with the risk of depression in a sample of middle-aged and older Finnish men, and this association remained significant even after adjusting for several potential confounders. The risk of depression was decreased by 47% for each 1 standard deviation increase in DGLA concentration (Model 3). CRP was not a predictor of depression and according to our results, the association between DGLA and depression was not dependent on inflammation. To the best of our knowledge, this is the first prospective study to report a significant inverse association between DGLA and the risk of depression.

The previous cross-sectional studies have shown contradictory results on the association between DGLA concentrations and prevalence of depressive symptoms (Chalut-Carpentier et al., 2015; Evans et al., 2012; Lotrich et al., 2013; Mamalakis et al., 2006). In morbidly obese subjects, depression scores were found to be inversely associated with DGLA concentration (Chalut-Carpentier et al., 2015). On the contrary, DGLA concentrations were positively associated with depressive symptoms in bipolar subjects (Evans et al., 2012), in hepatitis C patients treated with inflammatory cytokine interferon-alpha (Lotrich et al., 2013) and in healthy adolescents (Mamalakis et al., 2006). However, these studies demonstrated only cross-sectional associations, and none of these studies were conducted in an adult/elderly general population. Aging is known to affect metabolism of DGLA; it is a risk factor to a deficiency of DGLA (Wang et al., 2012). Moreover, DGLA concentrations have been demonstrated to be significantly higher in overweight

or obese individuals compared with normal-weight controls (Fekete et al., 2015). In our cohort, there was a significant association between baseline DGLA concentrations and depression, however, BMI did not attenuate the association.

The importance of DGLA in the management of depressive symptoms has yet to be elucidated. The monoamine hypothesis of depression is one possible mechanism behind the association between DGLA and depression. DGLA has been shown to play a role in monoamine system (Sinclair & Gibson, 1992). It is well-documented that both n-3 and n-6 PUFAs, including DGLA, are needed and involved in several physiological processes including membrane-bound enzymes and cellular signal transduction and cell membrane fluidity of the brain (Liperoti et al., 2009; Sinclair & Gibson, 1992). Neurotransmission in the normal nervous system can be regulated by the PUFAs mainly by altering the biophysical properties of cell membranes and the presynaptic vesicular release of classical amino acids and amine neurotransmitters (Heinrichs, 2010). A deficiency of DGLA in behavioural disorders could be mediated through dopaminergic systems, which play important roles in emotion regulation (Sinclair & Gibson, 1992). PGE1, one of the products of DGLA, has been shown to have antagonistic dopaminergic effects in many tissues (Sinclair & Gibson, 1992). This is noteworthy as many other systems involved in behavioural processes, such as the serotonergic, glutamatergic, gamma-aminobutyric acid (GABA)ergic, and cholinergic systems have interactions with dopaminergic systems (Chalon et al., 2001).

One of the aims in the present study was to assess if the association between DGLA and depression could be explained by inflammatory hypothesis of depression, as it is known that mental disorders, such as depression, are associated with the dysregulation of the inflammatory responses of the immune system. For example, higher levels of CRP associate directly and independently with depression (Howren et al., 2009). PUFAs, especially n-3 PUFAs, have been suggested to play a significant role in the prevention of depression via potential effects on inflammatory markers (Song, 2013). However, it is found that both n-3 and n-6 PUFAs may reduce inflammation, and DGLA has been found to be more effective in the controlling of inflammatory markers, including CRP, compared to EPA and DHA (Ristic-Medic et al., 2014). Despite the results described above, our study failed to detect evidence of an association between DGLA and CRP, known as the sensitive marker of inflammation. Thus, our results are not explained by inflammatory hypothesis of depression.

DGLA is the direct precursor of AA, however, conversion of DGLA to AA in humans is limited (Wang et al., 2012), and dietary intakes of pre-formed AA (from sources like red meat) are key determinants of blood concentrations of AA (Seah et al., 2017). Although we found the inverse association between DGLA concentrations and the risk of depression, AA concentrations were not associated with the risk of depression in our study population (Ruusunen et al., 2011). Further studies are needed to clarify the differences between different fatty acids on depression risk.

The population-based recruitment, using a hospital discharge diagnosis of depression, long follow-up, extensive examinations for potential confounders and the detailed information of depression events during the follow-up period are strengths of the current study. The weakness of this study is the use a single measurement of serum DGLA concentrations, which may attenuate the association during a long-follow-up. Since this study involved only middle-aged and older men from Eastern Finland, our findings might not be generalizable for women or other sub-populations. However, studying the association especially in aging population might be considered as a strength, as aging may affect DGLA metabolism (Wang et al., 2012). In addition, we were not able to assess mild depression subjects because of using a hospital discharge diagnosis of depression.

In conclusion, higher serum DGLA was associated with lower risk of depression. The mechanism of this association is unclear; therefore, further studies are warranted to find potential mechanisms of the association between DGLA and depression.

#### **Conflict of interests**

None.

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**Authorship**

The authors' responsibilities were as following: TY contributed to the interpretation of data and primary wrote the manuscript; TY performed the statistical analyses, TT, SML, T-PT, TN, JK, SV and AR contributed to the interpretation of data and writing of the manuscript; T-PT, TN and SV contributed to the data collection. TY and AR had primary responsibility for final content. All authors read and approved the final manuscript.

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**Table 1:** Baseline characteristics of the study subjects according to the hospital discharge diagnosis of depression.

Measure	Discharge diagnosis of depression (N=58)		No Depression (N=2043)		P
	Mean	SD	Mean	SD	
Age (years)	52.0	5.5	53.0	5.2	0.171
Education (years)	8.7	3.2	8.7	3.4	0.922

Alcohol (g/week)	68	81	69	113	0.933
Leisure-time physical activity (kcal/day)	112	108	143	176	0.189
BMI (kg/m <sup>2</sup> )	27.1	3.3	26.9	3.5	0.635
Total energy intake (Kcal/day)	2628	629	2434	607	0.017
C-reactive protein (mg/L)	1.52	1.61	1.82	1.74	0.183
Serum linoleic acid (%)	26.62	4.87	26.49	4.54	0.824
Serum gamma-linolenic acid (%)	0.29	0.13	0.29	0.11	0.940
Serum dihomo- $\gamma$ -linolenic acid (%)	1.26	0.24	1.34	0.31	0.042
Serum arachidonic acid (%)	4.72	1.09	4.78	1.01	0.638
Serum alpha-linolenic acid (%)	0.75	0.23	0.74	0.24	0.864
Serum eicosapentaenoic acid (%)	1.61	0.72	1.68	0.92	0.568
Serum docosahexaenoic acid (%)	2.39	0.72	2.47	0.74	0.465
Intake of fruits, berries and vegetables (g/day)	242	152	259	156	0.437
Intake of red meat (g/day)	170	81	138	75	0.001
	N	%	N	%	
Married (%)	50	86.2	1798	88.0	0.384
Smokers (%)	18	31.0	613	30.0	0.927

CVD (%)	7	12.0	361	18.0	0.267
Diabetes (%)	4	6.9	102	5.0	0.514

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases.

**Table 2: The hazard ratios of getting a hospital discharge diagnosis of depression according to serum concentration of dihomo- $\gamma$ -linolenic acid.**

Hospital discharge diagnosis of depression (N=58)			
HR	95 % CI	P	
0.69	0.51-0.94	0.020	Model 1
0.65	0.48-0.89	0.008	Model 2
0.53	0.36-0.79	0.002	Model 3
0.54	0.36-0.80	0.002	Model 3 + CRP

Standardized value of dihomo- $\gamma$ -linolenic acid was used in the model and, thus, for each 1 standard deviation increase in dihomo- $\gamma$ -linolenic acid the risk of depression is decreased by 47%.

Abbreviations: Confidence interval, CI; Hazards ratio, HR

**Model 1:** Age and examination year

**Model 2:** Adjusted for model 1+ smoking status (never, previous, current), marital status (married or living as a couple, not married, separated or divorced, widowed), education (years), alcohol intake (g/week), leisure-time physical activity (kcal/day), body mass index ( $\text{kg}/\text{m}^2$ ), total energy intake (Kcal/day), history of cardiovascular diseases (yes vs no), history of diabetes (yes vs no), history of mental illness (yes vs no)

**Model 3:** Adjusted for model 1, model 2 and linolenic acid (%), eicosapentaenoic acid (%), docosahexaenoic acid (%), alpha-linolenic acid (%), gamma-linolenic acid (%), arachidonic acid (%), CRP (mg/L) and depressive symptoms (continuous) at baseline.

### Highlights

- Serum dihomo- $\gamma$ -linolenic acid (DGLA) is an n-6 polyunsaturated fatty acid
- Prospective association between DGLA and risk of depression is unstudied
- Serum DGLA levels were inversely associated with risk of depression in elderly men
- Inflammation did not explain the observed association
- Potential mechanisms of the association need further investigation

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