Original article

Dietary and circulating fatty acids and ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition

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Abstract

Background. Fatty acids impact obesity, estrogens and inflammation, risk factors for ovarian cancer. Few epidemiological studies have investigated the association of fatty acids with ovarian cancer.

Methods. Within the European Prospective Investigation into Cancer and nutrition, 1,486 incident ovarian cancer cases were identified. Cox Proportional Hazard models with adjustment for ovarian cancer risk factors were used to estimate hazard ratios of ovarian cancer across quintiles of intake of fatty acids. False discovery rate was computed to control for multiple testing. Multivariable conditional logistic regression models were used to estimate odds ratios of ovarian cancer across tertiles of plasma fatty acids among 633 cases and two matched controls in a nested case-control analysis.

Results. A positive association was found between ovarian cancer and intake of industrial *trans* elaidic acid (Hazard Ratio comparing 5^{th} with 1^{st} quintile$_{Q5\text{-}Q1}$=1.29; 95% CI=1.03-1.62; p_{trend}=0.02, q-value=0.06). Dietary intakes of *n*-6 linoleic acid ($HR_{Q5\text{-}Q1}$=1.10; 95% CI=1.01-1.21; p_{trend}=0.03) and *n*-3 α-linolenic acid ($HR_{Q5\text{-}Q1}$=1.18; 95% CI=1.05-1.34; p_{trend}=0.007) from deep frying fats were also positively associated with ovarian cancer. Suggestive associations were reported for circulating elaidic (Odds Ratio comparing 3^{rd} with 1^{st} tertile$_{T3\text{-}T1}$ = 1.39; 95% CI=0.99–1.94; p_{trend}=0.06) and α-linolenic acids ($OR_{T3\text{-}T1}$=1.30; 95% CI=0.98–1.72; p_{trend}=0.06).

Conclusion. Our results suggest that higher intakes and circulating levels of industrial *trans* elaidic acid, and higher intakes of linoleic acid and α-linolenic acid from deep frying fat, may be associated with greater risk of ovarian cancer.

Impact. If causal, eliminating industrial *trans* fatty acids could offer a straightforward public health action for reducing ovarian cancer risk.

Introduction

Ovarian cancer, with 295,414 new cases and 184,799 deaths in 2018 worldwide, is the eighth most common cancer and the eighth most common cause of cancer death in women [1]. As the incidence of ovarian cancer is rising worldwide, prevention strategies are urgently needed; however, few preventable factors have been identified [2]. Data mainly derived from case-control studies suggest that a typical Western diet, high in fats and meats and low in vegetables, might be associated with a higher risk of Epithelial Ovarian Cancer (EOC) [3].

A systematic meta-analysis by the World Cancer Research Funds concluded there was "limited" evidence for a link between saturated/animal fat and *trans* fatty acids and EOC risk [4]. Data from the European Prospective Investigation into Cancer and Nutrition (EPIC) and the Netherlands Cohort Studies reported a greater risk of EOC associated with a higher intake of saturated fat [5]. In the EPIC study only, higher intake of polyunsaturated fatty acids was associated with higher risk of EOC[6]. Finally, higher fat intake from animal sources, but not from plant sources, was associated with a greater risk of EOC in the National Institutes of Health-American Association of Retired Persons (NIH-AARP) diet and health study [7].

The aim of this study was to prospectively investigate the association between individual fatty acids intake from various food sources as well as circulating biomarker levels and EOC risk in the EPIC study.

Materials and methods

Study design

The EPIC study includes 521,330 participants recruited between 1992 and 2000 from 23 centers across 10 European countries [8]. The study design, recruitment procedures and data collection have been described previously [9]. Briefly, dietary information, as well as socio-demographic, and lifestyle data were collected at enrolment from all participants by administration of country-specific questionnaires.

Baseline anthropometric measurements and peripheral blood samples were also collected. Procedures for sample collection, processing and storage are described in detail elsewhere [10].

From a total of 333,224 women enrolled in the EPIC study, women were excluded from the current analysis if they did not complete a lifestyle or dietary questionnaire (n = 3,243), or were classified in the top or bottom 1% of energy intake to energy requirement (n = 6,467), leaving 323,514 eligible women.

Informed consent was provided by all participants, and ethical approval for the study was obtained from the internal review board of the International Agency for Research on Cancer (IARC) and from local ethics committees in each participating country.

Assessment of dietary fatty acids intake

To compile the EPIC Nutrient Database (ENDB) for the EPIC study, a highly-standardised procedure was used, adopting nutrient values from ten national food composition databases of the respective EPIC countries [11, 12]. ENDB was used to match the EPIC data with fatty acid isomers using the National Nutrient Database for Standard Reference of the United States (NNDSR; further referred to as USDA table) [13]. A follow-up validation of the EPIC food frequency questionnaire using two repeated dietary questionnaires and 12 consecutive monthly

24-hour dietary recalls showed that intakes of fats and other nutrient/food items reported at recruitment across countries were reliable over time[14]. For example, the Spearman correlation coefficients reported for different types of fat intakes ranged between 0.14 and 0.75 in men and, 0.30 and 0.73 in women. In another validation study within the EPIC cohort, Spearman's correlation coefficients between the intake of saturated, monounsaturated, and polyunsaturated fat estimated through a self-administered 20-item short questionnaire and the FFQ were 0.50, 0.43 and 0.29 ($p<0.01$), respectively[15]. In addition, the reliability of fatty acid composition measured in human blood phospholipid by gas chromatography was assessed between three independent measurements of blood fatty acids in the Nurses' Health Study (NHS)[16]. The correlation coefficients between three measures over a 2 years period were greater than 0.50 for most fatty acids, including *trans* fatty acids[16]. These findings suggested that a single determination of dietary estimates and circulating phospholipid fatty acids can be acceptable.

Quality control was tested through the comparison of the nutrients included in the extended EPIC database with nutritional biomarkers available in the nested case-control studies in EPIC (e.g. correlation between *trans*-fatty acids derived from the dietary questionnaires and the fatty acids extracted from plasma phospholipids was 0.53).

Ascertainment of ovarian cancer cases

Incident EOC were identified through population-based cancer registries or active follow-up. EOC were classified as ovarian, fallopian tube, and primary peritoneal cancers based on the third revision of the International Classification of Diseases for Oncology codes C56.9, C57.0 and C48, respectively.

Among 323,514 women enrolled in the EPIC study, 1,624 first-incident EOC were identified after a mean follow-up of 8.2 years. Cases were censored if they were non-epithelial (n

=76), or tumors of borderline malignancy (n =62), leaving 1,486 EOC cases for the current analysis. Cancer end point data is based on the latest round of follow-up received from the EPIC centres and centralized at IARC between 2014-2016. For each EPIC study centre, closure dates of the study period were defined as the latest dates of complete and verified follow-up for both cancer incidence and vital status (dates varied between centers, between June 2008 and December 2013).

Nested case-control study and analysis of plasma phospholipid fatty acids

A total of 1,075 cases of first incident invasive EOC were identified among women who had completed the dietary questionnaire and provided a baseline blood sample. Samples from Denmark were not included in this analysis, resulting in 633 cases. For each case, two controls were randomly selected from female cohort members who were alive, had blood samples available, had no bilateral ovariectomy and were cancer-free at diagnosis of the matched case, using a sampling protocol described previously [17]. Controls were matched to cases on study center, age at blood donation (±1year), time of the day of blood collection, fasting status, menopausal status, and menstrual cycle phase for premenopausal women, current use of oral contraceptives or hormonal replacement therapy (HRT).

Analysis of plasma phospholipid fatty acids

The methodology used to determine plasma phospholipid concentrations of sixty fatty acids from short-chain SFA to long-chain PUFA, including fifteen *trans* fatty acid isomers from industrial processes and animal sources, has been previously described [18]. Samples from cases and controls were processed in the same batch, and laboratory staff was blinded to case-control status and quality controls. The relative amount of each fatty acid was expressed as percentage of total fatty acids and as absolute amount (μmol/l).

The coefficients of variation (CV) were calculated using two quality control samples within each batch. Overall CV (intra- and inter-assays) ranged from 0.013% for large peaks (16:0) to 9.34% for the smallest peaks (18:3*n*-3ctt). All laboratory analyses were performed at IARC.

Using values for 60 individual fatty acids, we calculated the percentage of the following groups: SFA, *cis* MUFA, rTFA, iTFA, *cis n*-6 PUFA (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5), and *cis n*-3 PUFA. We calculated the ratio of long-chain *n*-6/long-chain *n*-3 PUFA. We also determined the desaturation indexes $DI_{16\ and}\ DI_{18}$ as biomarkers of endogenous lipogenesis of MUFA [19].

Statistical analyses

The socio-demographic and lifestyle characteristics as well as dietary intake of fatty acids were represented in terms of frequencies for the categorical variables and means ± standard deviations (SD) for the continuous variables. Cox Proportional Hazards regression using age as the underlying time metric with the subjects' age at recruitment as the entry time and their age at cancer diagnosis (except for non-melanoma skin cancer), death, emigration or last complete follow-up, whichever occurred first, as the exit time was used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) for the association between dietary fatty acids and EOC risk. Intakes of fatty acids were divided into quintiles based on their distribution in all cohort participants at baseline, setting women in the lowest category of fatty acids intake as the reference group. All models were stratified by the study center and age at enrolment. The final multivariable model retained was adjusted for duration of oral contraceptive use (never use; use <5 years; use ≥5 years; missing), parity (number of live born and/or still born children; 0, 1-2, 3-4; >4; missing), menopausal status at enrolment (premenopausal; postmenopausal; perimenopausal/unknown menopause) and total energy intake (continuous). Additional potential confounders including

history/duration of breastfeeding, ever use of postmenopausal hormones, history of unilateral ovariectomy, BMI, physical activity, smoking status, level of education, intake of alcohol, red meat, and total sugar were not included in the final models as they did not alter the relative risk estimates by 10% (data not shown). In addition, mutual adjustment of fatty acids for each other did not modify the risk estimates (data not shown). Tests for trend were computed using the quintile specific median of each fatty acid.

Due to the number of tests performed, q-values were calculated using the false discovery rate of the Benjamini-Hochberg procedure [20].

Additionally, associations between individual fatty acid intakes (as continuous variables) and EOC risk were investigated by their dietary sources when relevant. The main dietary sources of individual fatty acid were selected when they contributed to more than 1% of their dietary intakes. The percentage of contribution was calculated for each food (sub-) group based on the mean daily intake reported in the questionnaire. The population proportion formula was used to determine the percentage contribution of each food group to the intake of each fatty acid component. This was done by summing the amount of the component provided by the food for all individuals divided by the total intake of that component from all foods for the entire study population.

The population attributable fraction (PAF) for fatty acids was estimated using the following equation which uses the prevalence of fatty acid's exposure as categorical variable and the associated relative risk (or Hazard Ratio) in the current cancer cases:

$$PAF = \frac{\sum_{i=1}^{k} RR_i\, p_i - \sum_{i=1}^{k} RR_i\, p_i^*}{\sum_{i=1}^{k} RR_i\, p_i},$$

With RR_i and p_i expressing the adjusted hazard ratio and the observed proportion of participants in category i, and p_i* the counterfactual proportion of participants [21]. Given the low EOC prevalence and under the proportional hazards assumption, HR were correct approximations of risk ratios (RR_i). Confidence intervals were obtained using bootstrap sampling [22].

Plasma phospholipid fatty acid values were log-transformed and geometric means with 95% CI were calculated. Plasma phospholipid fatty acid values were divided into tertiles based on the distribution among the controls. Conditional logistic regression models were used to evaluate the association between plasma phospholipid fatty acids and EOC. Models were adjusted for the same confounders as those selected above for the analyses on dietary intakes.

Cox Proportional Hazards competing risks analysis [23] was used to estimate separate HR and 95% CI for serous, mucinous, endometrioid and clear cell EOC, and for grade 1, 2 and 3. Further analyses stratified by menopausal status, parity, BMI (<18.5; 18.5-24.9; 25.0 –29.9; ≥30.0), and time from blood collection to diagnosis (<5 years; ≥5 years) were performed. Formal tests of heterogeneity were based on chi-square statistics, calculated as the deviations of logistic beta-coefficients observed in each of the subgroups relative to the overall beta-coefficient.

To limit bias due to reverse causation, sensitivity analyses excluding cases diagnosed during the first 2 years of follow-up were also conducted.

All statistical analysis were carried out using STATA 14.0 (StataCorp, College Station, TX, USA). P-values below 0.05 were considered statistically significant.

Results

Compared to the non-cases, the EOC cases were more likely to have a higher BMI, be nulliparous, post-menopausal, to have ever used HRT, to have a lower education, and were less likely to have ever used oral contraceptives. In the nested case-control analysis, cases were more likely to be nulliparous, and were less likely to have ever used oral contraceptives (**Table 1**).

A positive association was found between EOC risk and intakes of iTFA (HR comparing 5th with 1st quintile$_{Q5\text{-}Q1}$=1.34, 95% CI=1.06-1.67, p_{trend}=0.01, q-value=0.04) mainly driven by elaidic acid ($HR_{Q5\text{-}Q1}$= 1.29; 95% CI=1.03-1.62; ptrend=0.02, q-value=0.06). A positive association was also reported between EOC risk and intakes of total PUFA ($HR_{Q5\text{-}Q1}$=1.41, 95% CI=1.13-1.77, p_{trend}<0.001, q-value=0.005), mainly driven by linoleic acid ($HR_{Q5\text{-}Q1}$=1.34, 95% CI=1.07-1.67, p_{trend}<0.001, q-value=0.005), and α-linolenic acid ($HR_{Q5\text{-}Q1}$=1.29, 95% CI=1.05-1.58, p_{trend}=0.007, q-value=0.002) (**Table 2**). PAF estimate indicated that 11.7% (95% CI (1.9%, 27.4%)) of EOC risk can be attributed to *trans* elaidic acid..

A borderline positive trend was reported between EOC risk and plasma phospholipid elaidic acid (OR comparing 3rd with 1st tertile$_{T3\text{-}T1}$=1.39, 95% CI=0.99-1.94, p_{trend}=0.06) but not with plasma phospholipid iTFA despite the high correlation between the individual elaidic and the total iTFA (Spearman's rho=0.88, p<0.001). A borderline positive trend was also reported between EOC risk and plasma phospholipid α-linolenic acid ($OR_{T3\text{-}T1}$=1.30, 95% CI= 0.98-1.72, p_{trend}= 0.06) (**Table 3**).

The overall positive association between linoleic acid and EOC risk was mainly driven by the contribution of deep-frying fat ($HR_{Q5\text{-}Q1}$=1.10, 95%CI=1.01-1.21) (**Figure 1**). In contrast, an inverse association was found between linoleic acid from vegetable oils and EOC risk ($HR_{Q5\text{-}}$

$_{Q1}$=0.97, 95%CI=0.95-0.99) (Figure 1). The overall positive association between α-linolenic acid and EOC risk was mainly driven by the contribution of deep-frying fat (HR_{Q5-Q1}=1.18, 95%CI=1.05-1.34) and margarine (HR_{Q5-Q1}=1.02, 95%CI=1.01-1.04) (**Figure 2**).

Finally, the association between fatty acids and EOC did not vary according to histological subtypes of EOC or grade (data not shown). Stratified analysis by BMI, parity and lag time did not show any substantial differences in the risk estimates (data not shown). All p for heterogeneity >0.05. Stratified analysis by menopausal status showed a positive association between palmitic acid and EOC risk restricted to premenopausal women (HR_{Q5-Q1}=2.13, 95% CI=1.22-3.71), while no association was found in postmenopausal women ($p_{heterogeneity}$ = 0.04).

Discussion

To our knowledge, this is the first prospective analysis of the association between dietary and circulating individual fatty acids and the risk of EOC. We found evidence of a higher risk of EOC associated with higher dietary intakes of *trans* elaidic acid, linoleic acid and α-linolenic acid. Suggestive positive associations were reported for plasma phospholipid *trans* elaidic acid and α-linolenic acid. These associations did not vary according to histological subtypes of EOC.

iTFA consumption is associated with increased all-cause mortality [24] and the WHO encourages the elimination of these fatty acids from the diet [25]. TFA may have decreased in processed foods, but their intake may still be high in certain countries or vulnerable groups in the population [26]. In our study, dietary intake of elaidic acid, the main iTFA was significantly positively associated with EOC risk, and risk increased at dietary intakes of iTFA below dietary limits of 1% recommended by WHO. Similarly, in our subset analysis, we found a borderline significant positive association between plasma phospholipid *trans* elaidic acid and EOC risk but not with plasma phospholipid iTFA. One case-control study conducted in New England reported a significant association between higher intake of *trans* fat and greater risk of EOC [27]. These data need further replication and clarification but suggest that iTFA from industrial processes, even at low intakes, might increase EOC development. In the current study, PAF estimate indicated that 11.7% (95% CI (1.9%, 27.4%)) of EOC risk can be attributed to industrial *trans* elaidic acid. Assuming the estimated HR between elaidic acid and EOC risk is a good approximation of the causal relative risk, a total of 173 cases (range (28 cases, 407 cases)) could have been avoided in the population study if elaidic acid was removed from diet. As already reported in the EPIC [6] and the NIH-AARP Diet and Health studies [7], we found a positive association between intake of total PUFA and EOC risk. In the current analysis, available data on individual fatty acids indicated that

this positive association is mainly driven by linoleic acid and α-linolenic acid, essential PUFA of the *n*-6 and *n*-3 families, respectively. In contrast, no association was reported between intakes of linoleic and α-linolenic acid and EOC risk in the Nurses' Health NHS [28]. These disparities between the NHS study and our study might be due to differences in the number of cases between the two studies (301 cases in the NHS vs 1486 in the current study). The possibility that these differences might be due to different intakes of these fatty acids or different dietary contributors in the two populations is not known but deserve further consideration.Our results were further confirmed by a positive trend between plasma phospholipid levels of alpha-linolenic acid and EOC risk in our subset analysis of the EPIC study, but not with plasma linoleic acid. This might be due to a higher endogenous conversion of linoleic acid to long-chain n-6 polyunsatyurated fatty acids compared with the limited conversion of alpha-linoleic acid to its longer chain derivatives[29].

In contrast to iTFA including elaidic acid which are derived from processed foods and deep frying fat only, linoleic and α-linolenic acids have various food sources, vegetable, animal and industrial contributing to their daily intakes. However, we found divergent associations between linoleic and α-linolenic acids and EOC according to their dietary sources. The positive association between linoleic acid and EOC risk is only significantly driven by deep frying fat, even if deep frying fat is a minor contributor to linoleic acid (0.28%). Other positive trends with linoleic acid from fruit, nuts and seeds, eggs and eggs products and total fat were reported, but not significant. In contrast, an inverse association was found between linoleic acid from vegetable oils and EOC risk. Regarding α-linolenic acid, the positive association with EOC is mainly driven by deep frying fat and margarine. Other positive trends with α-linoleic acid from cereal and cereal products, meat and meat products, fat, sugar and confectionaries, cakes and biscuits and condiments and sauces, were reported but are not significant.

These data might suggest that linoleic and α-linolenic acids may not exert a direct effect on EOC development which might be rather associated to co-exposure to other potentially carcinogenic compounds occurring in foods exposed to deep frying fat, such as aldehydes, oxidized lipids, heterocyclic compounds, *trans* fatty acids, polymers, sterol derivatives, acrylamide, and acrolein [30].

Our study has several strengths including its prospective design, and a very large number of incident EOC cases. In addition, having information from both dietary estimates and circulating fatty acids allowed the comparison of these independent approaches. Additionally, we were able to separate *n*-6 and *n*-3 *cis* PUFA isomers as well as *trans* fatty acid isomers from natural and industrial processes in both food composition table and plasma phospholipids. The major limitation of the study is the single collection of questionnaires and blood samples at baseline.. Another limitation was that we did not have data for ovariectomy conducted during follow-up.

Conclusion

Our results suggest that higher dietary intakes and circulating levels of industrial *trans* elaidic acid, along with higher intakes of linoleic acid and α-linolenic acid originating mainly from deep frying fat, may be associated with greater risk of EOC. If causal, eliminating elaidic acid through a regulation on industrial processes and limiting their use as deep frying fat could potentially offer a relatively straightforward public health action for reducing EOC risk.

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Table 1. Characteristics of the study population

EPIC-wide study	Epithelial ovarian cancer cases	Non cases*	**Nested case-control study**	Epithelial ovarian cancer cases	Controls	
N= 323,376	n=1,486	n=321,890		n=633	n=1,248	p**
Anatomical subtypes, number, (%*)**						
Serous	79 (53.4)	-		341 (53.7)	-	-
Mucinous	91 (6.1)	-		37 (5.8)	-	-
Endometroid	135 (9.1)	-		69 (10.8)	-	-
Clear cell	68 (4.6)	-		23 (3.6)	-	-
Follow-up characteristics Mean±SD***						
Age at recruitment, years	54.7±8.2	50.6±9.8		54.7±8.8	54.6±8.8	matched
Age at diagnosis, years	62.9±9.8	-		62.6±9.3	-	-
Follow-up, years	8.2 ±4.7	13.9±3.8		7.9±4.5	14.7±2.6	**<0.001**
Anthropometry Mean±SD***						
Weight, kg	67.3±12.2	65.6±11.6		67.7±11.7	66.6±11.7	0.14
Height, cm	162.5±6.6	162.3±6.7		160.1±6.9	160.1±6.7	0.83
BMI, $kg.m^{-2}$	25.5±4.5	24.9±4.4		26.3±4.6	25.9±4.6	0.08
Obese (BMI ≥30 kg/m^2), %***	14.8	12.3		19.9	17.5	0.20
Reproductive and hormone factors						
Number of full term pregnancies#	1.9±1.2	1.9±1.2		1.9±1.3	2.1±1.3	**<0.01**
Nulliparous ,%	16.3	14.1		16.2	12.3	**0.02**
Ever use Oral contraceptives, %***						**<0.01**
Never	53.1	40.7		58.8	50.9	
Ever	46.9	59.3		41.2	49.1	

Ever use hormone replacement therapy##,%***					0.84
Never	67.3	75.2	74.6	74.2	
Ever	32.7	24.8	25.4	25.8	
Ever breastfed#, %***					0.10
No	28.6	27.8	28.4	24.8	
Yes	71.4	72.2	71.6	75.2	
Ovariectomy, %***					**<0.01**
No	97.6	95.8	98.4	95.4	
Unilateral	2.4	4.2	1.6	4.6	
Menopausal Status,%***					matched
Premenopausal	20.8	36.0	25.7	24.9	
Post menopausal	61.2	44.4	59.9	59.5	
Perimenopausal	17.9	19.6	14.4	15.5	
Age at menopause##	49.6±4.7	48.9±4.8	49.7±4.5	49.1±4.7	0.07
Socio-economic status and lifestyle					
Total energy intake, Kcal/day	1959.1±527.9	1991.6±545.4	2002.1±540.3	1993.1±514.4	0.73
Alcohol intake, %***					**0.01**
None	7.7	6.7	8.8	7.1	
<5g/day	49.9	48.9	56.8	52.2	
5 to <14.9 g/day	26.4	27.3	20.2	25.9	
15.0 to <29.9 g/day	10.7	11.0	10.9	9.6	
≥29.9 g/day	5.4	6.1	3.4	5.3	
Education status, %					0.66
None and primary school	31.9	27.8	40.6	40.5	
Technical or professional and secondary school	43.0	45.3	35.4	36.8	
Higher education	18.9	23.0	17.1	16.9	
Physical activity status, %***					0.52
Inactive	12.0	13.1	8.4	7.1	

Moderately inactive	31.3	33.0		23.4	25.9	
Moderately active	47.2	44.2		56.9	55.2	
Active	9.5	9.7		11.2	11.8	
Smoking status, %***						0.65
Never	54.1	56.9		59.3	61.3	
Former	26.3	23.1		22.6	22.1	
Current	19.6	20.0		18.1	16.6	
Dietary intake, (g/day) Median (95%CI)* **						
Dairy products	295.5 (50.6-781.5)	277.9 (51.1-720.7)		297.8 (49.1-751.8)	301.2 (46.9-733.1)	0.73
Cereal and cereal products	176.1 (75.9-365.7)	187.4 (77.0-386.9)		181.6 (73.5-376.3)	183.8 (80.9-383.9)	0.58
Meat and meat products	80.9 (4.9-166.1)	83.1 (2.4-178.0)		83.5 (8.9-163.4)	87.5 (15.1-171.2)	0.28
Fat	22.8 (5.1-55.5)	22.1 (5.5-53.5)		25.6 (6.9-59.4)	25.4 (5.7-54.8)	0.25
Vegetable oils	2.9 (0.0-36.9)	3.8 (0.0-39.7)		5.9 (0.1-50.1)	5.9 (0.2-47.9)	0.70
Butter	0.2 (0.0-22.3)	0.4 (0.0-21.0)		0.4 (0.0-23.1)	0.5 (0.0-20.2)	0.50
Margarine	7.7 (0.0-40.4)	4.3 (0.0-35.9)		2.7 (0.0-32.7)	2.2 (0.0-29.3)	0.26
Deep frying fat	0.0 (0.0-1.4)	0.0 (0.0-1.5)		0.0 (0.0-2.6)	0.0 (0.0-2.4)	0.52
Cakes and biscuits	29.6 (1.1-125.3)	29.7 (0.1-112.3)		33.3 (0.0-14.6)	31.3 (0.0-125.3)	0.12
Sugar and confectionaries	30.0 (3.6-98.4)	28.7 (2.4-97.2)		26.7 (2.0-86.7)	27.0 (2.3-87.6)	0.36
Condiments and sauces	15.2 (0.9-56.6)	15.4 (0.9-55.9)		12.3 (0.1-51.5)	13.0 (0.4-50.1)	0.41
Fatty acid intake### (g/day or mg/day) Median (95%CI)* **			**Phospholipid fatty acids### (% of total fatty acids) Mean±SD*** **			
SFA (g/day)	24.0 (11.8-46.3)	24.9 (11.7-48.3)	SFA	40.8±1.7	40.9±1.3	0.30

Cis MUFA (g/day)	23.3 (11.6-45.6)	24.6 (12.1-48.1)	Cis MUFA	12.9±2.1	12.9±2.0	0.95
rTFA (mg/day)	23.4 (3.8-123.9)	28.2 (4.3-134.6)	rTFA	0.4±0.2	0.4±0.2	0.34
iTFA (g/day)	1.4 (0.2-5.2)	1.2 (0.2-4.8)	iTFA	0.7±0.3	0.7±0.4	0.94
n-6 PUFA (g/day)	11.5 (5.6-21.3)	11.4 (5.8-22.4)	*n*-6 PUFA	37.6±3.3	37.5±3.3	0.44
Linoleic acid (g/day)	11.5 (5.6-21.3)	11.4 (5.7-22.3)	Linoleic acid	22.5±3.4	22.4±3.4	0.35
n-6 long-chain PUFA (mg/day)	23.5 (6.5-66.2)	24.2 (5.7-66.2)	*n*-6 long-chain PUFA	15.0±2.5	15.0±2.5	0.78
n-3 PUFA (mg/day)	729.4 (258.7-2066.4)	665.4 (237.7-1907.2)	*n*-3 PUFA	7.3±2.3	7.3±2.3	0.54
α-linolenic acid (mg/day)	421.2 (122.7-1326.5)	383.1 (117.5-1252.1)	α-linolenic acid	0.2±0.1	0.2±0.1	0.47
n-3 long-chain PUFA (mg/day)	208.3 (24.5-1129.2)	196.8 (21.9-1037.9)	*n*-3 long-chain PUFA	7.1±2.1	7.1±2.3	0.52

*Considered as non-cases at the most recent cancer endpoint and vital status update

**Student's t-test for continuous variables and Chi2 test for categorical variables in the nested case-control approach

***Continuous variables are presented as means and standard deviations (SD) or median (95%CI). Categorical variables are presented as percentages. Missing values were excluded from percentage calculations

#Among parous women
##Among postmenopausal women only
###Groupings of fatty acids are as described in Materials and Methods

Table 2. Association of estimated dietary intakes of fatty acids with ovarian cancer risk in the EPIC cohort

	Q1	Q2	Q3	Q4	Q5	P trend†	q trend§
	Reference						
Total SFA[a]							
Mean Intake ±SD (g/d)	13.51±2.80	19.90±1.47	25.03±1.54	31.15±2.11	44.60±9.13		
Cases/non-cases (n)	329/64,342	303/64,368	326/64,344	269/64,402	259/64,411		
HR (95% CI)[#] *	1.00	0.93 (0.79;1.10)	1.10 (0.92;1.32)	0.96 (0.78;1.18)	1.12 (0.87;1.44)	0.44	0.60
Palmitic acid (16:0)							
Mean Intake ±SD (g/d)	7.51±1.45	10.77±0.74	13.30±0.75	16.25±0.99	22.48±4.15		
Cases/non-cases (n)	341/64,331	299/64,371	329/64,341	250/64,421	267/64,403		
HR (95% CI)*	1.00	0.92 (0.78;1.09)	1.08 (0.90;1.30)	0.89 (0.72;1.11)	1.13 (0.87;1.48)	0.63	0.78
Odd chain saturated fatty acids[b]							
Mean Intake ±SD (mg/d)	50.00±20.00	90.00±10.00	140.00±10.00	200.00±20.00	340.00±140.00		
Cases/non-cases (n)	334/64,337	309/64,363	267/64,403	301/64,369	275/64,395		
HR (95% CI)*	1.00	1.03 (0.87;1.22)	0.93 (0.78;1.12)	1.07 (0.89;1.30)	1.12 (0.90;1.39)	0.31	0.49
Total *cis* MUFA[c]							
Mean Intake ±SD (g/d)	13.76±2.63	19.77±1.39	24.65±1.47	30.642.11	44.32±9.44		
Cases/non-cases (n)	346/64,325	326/64,345	295/64,375	285/64,386	234/64,436		
HR (95% CI)*	1.00	1.04 (0.89;1.24)	1.02 (0.85;1.24)	1.15 (0.92;1.43)	1.15 (0.86;1.53)	0.27	0.45
Oleic acid (18:1*n*-9)							
Mean Intake ±SD (g/d)	12.72±2.44	18.34±1.32	23.02±1.41	28.78±2.01	42.04±9.21		
Cases/non-cases (n)	342/64,329	341/64,331	286/64,383	282/64,389	235/64,435		
HR (95% CI)*	1.00	1.14 (0.96;1.34)	1.02 (0.85;1.25)	1.18 (0.95;1.48)	1.19 (0.89;1.60)	0.25	0.45

Total ruminant *trans* fatty acids[d]							
Mean Intake ±SD (mg/d)	6.00±3.00	15.00±3.00	29.00±5.00	52.00±8.00	120.00±57.00		
Cases/non-cases (n)	382/64,289	293/64,381	277/64,390	291/64,382	243/64,425		
HR (95% CI)*	1.00	0.96 (0.80;1.14)	0.94 (0.78;1.15)	1.03 (0.84;1.27)	1.01 (0.81;1.27)	0.67	0.78
Total industrial *trans* fatty acids[e]							
Mean Intake ±SD (g/d)	0.30±0.14	0.74±0.12	1.21±0.15	1.93±0.28	4.18±1.67		
Cases/non-cases (n)	234/64,437	255/64,416	286/64,384	323/64,348	388/64,282		
HR (95% CI)*	1.00	1.16 (0.95;1.41)	1.23 (0.99;1.51)	1.29 (1.04;1.60)	1.34 (1.06;1.67)	**0.01**	**0.04**
Elaidic acid (18:1*n*-9/12)							
Mean Intake ±SD (g/d)	0.27±0.13	0.69±0.12	1.14±0.15	1.85±0.28	4.11±1.67		
Cases/non cases (n)	238/64,433	254/64,417	280/64,390	323/64,348	391/64,279		
HR (95% CI)*	1.00	1.13 (0.93;1.37)	1.17 (0.95;1.44)	1.24 (1.01;1.54)	1.29 (1.03;1.62)	**0.02**	0.06
Total *cis n*-6 PUFA[f]							
Mean Intake ±SD (g/d)	6.51±1.17	9.21±0.63	11.42±0.67	14.17±0.97	20.69±4.81		
Cases/non-cases (n)	308/64,363	271/64,400	283/64,387	327/64,344	297/64,373		
HR (95% CI)*	1.00	0.99 (0.83;1.17)	1.13 (0.94;1.36)	1.32 (1.09;1.60)	1.33 (1.06;1.67)	**0.001**	**0.005**
Linoleic acid (18:2*n*-6)							
Mean Intake ±SD (g/d)	6.48±1.16	9.17±0.63	11.38±0.67	14.13±0.97	20.64±4.80		
Cases/non-cases (n)	309/64,362	270/64,401	280/64,390	329/64,342	298/64,372		
HR (95% CI)*	1.00	0.99 (0.83;1.17)	1.11 (0.93;1.33)	1.37 (1.13;1.66)	1.34 (1.07;1.67)	**<0.001**	**0.005**
Total long-chain *n*-6 PUFA[g]							
Mean Intake ±SD (mg/d)	8.00±3.00	17.00±2.00	24.00±2.00	34.00±4.00	61.00±24.00		
Cases/non-cases (n)	287/64,384	337/64,348	278/64,384	288/64,377	296/64,374		

HR (95% CI)*	1.00	1.08 (0.91;1.29)	1.14 (0.95;1.37)	1.24 (1.01;1.51)	1.21 (0.97;1.50)	0.42	0.60
Total cis *n*-3 PUFA[h]							
Mean Intake ±SD (g/d)	0.29±0.08	0.49±0.05	0.67±0.06	0.93±0.10	1.70±0.61		
Cases/non-cases (n)	252/64,420	273/64,397	281/64,389	308/64,363	372/64,298		
HR (95% CI)*	1.00	1.09 (0.91;1.31)	1.12 (0.93;1.36)	1.12 (0.91;1.37)	1.15 (0.93;1.43)	0.25	0.45
α-linolenic acid (18:3*n*-3)							
Mean Intake ±SD(g/d)	0.15±0.05	0.27±0.03	0.38±0.04	0.56±0.07	1.10±0.44		
Cases/non-cases (n)	260/64,411	262/64,409	278/64,393	306/64,364	380/64,290		
HR (95% CI)*	1.00	1.01 (0.84;1.21)	1.10 (0.92;1.33)	1.17 (0.97;1.42)	1.29 (1.05;1.58)	**0.007**	**0.002**
Total long-chain *n*-3 PUFA[i]							
Mean Intake (mg/d)	40.00±21.00	110.00±21.00	200.00±27.00	340.00±60.00	920.00±604.00		
Cases/non-cases (n)	273/64,398	293/64,378	276/64,395	300/64,370	344/64,326		
HR (95% CI)*	1.00	1.052 (0.86;1.22)	0.95 (0.78;1.14)	1.02 (0.84;1.24)	0.96 (0.78;1.19)	0.76	0.81
Total *cis* PUFA[j]							
Mean Intake ±SD (g/d)	7.05±1.23	9.88±0.66	12.20±0.70	15.07±1.01	21.87±5.01		
Cases/non-cases (n)	300/64,371	268/64,403	278/64,392	330/64,341	310/64,360		
HR (95% CI)*	1.00	0.99 (0.84;1.19)	1.12 (0.94;1.34)	1.39 (1.15;1.68)	1.41 (1.13;1.77)	**<0.001**	**0.005**
Ratio *n*-6/*n*-3 PUFA							
Mean Intake ±SD	7.80±2.29	13.08±1.24	17.47±1.34	23.12±2.06	38.91±28.11		
Cases/non-cases (n)	405/64,266	299/64,372	262/64,408	279/64,392	241/64,429		
HR (95% CI)*	1.00	0.87 (0.74;1.03)	0.90 (0.75;1.08)	1.03 (0.86;1.25)	0.92 (0.75;1.13)	0.99	0.99

 # HR = hazard ratio; CI = confidence interval

† *P or q values < 0.05 are shown in boldface type*

§ *Value for FDR (False Discovery Rate) correction*

** Stratified by study center and age (in one-year categories), and adjusted for total duration of oral contraceptive use, parity, menopausal status, and total energy intake*

[a]Total SFA included 4:0,6:0, 8:0,10:0,12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; [b]Odd chain fatty acids included 15:0, 17:0; [c]Total cis MUFA included 16:1n-7/n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1;

[d]Total trans ruminant fatty acids included 18:1n-7t, CLA; [e]Total trans industrial fatty acids included 16:1n-9t, 18:1n-9t, 18:2n-6tt, 18:3n-3ttt; [f]Total n-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4; [g]Total long-chain n-6 PUFA included 20:2, 20:3, 20:4; [h]Total n-3 PUFA included 18:3, 20:3, 20:5, 22:5, 22:6; [i]Total long-chain n-3 PUFA included 20:3, 20:5, 22:5, 22:6; [j]Total cis-PUFA included total n-6PUFA and total n-3 PUFA.

Table 3: Association of plasma phospholipid fatty acids with ovarian cancer risk in the EPIC cohort

	Tertile 1	Tertile 2	Tertile 3	P trend†
Plasma phospholipid fatty acids (% of total fatty acids)	Reference	OR (95%CI)	OR (95%CI)	
Total SFA[a]				
Mean±SD	39.58±1.27	40.91±0.25	42.20±0.94	
Cases/controls (n)	233/418	185/416	215/414	
OR (95% CI)[#]*	1.00	0.82 (0.64;1.06)	0.93 (0.70;1.23)	0.57
Palmitic acid (16:0)				
Mean±SD	24.08±1.57	25.88±0.42	27.90±1.12	
Cases/controls (n)	222/419	224/418	187/411	
OR (95% CI)*	1.00	0.94 (0.73;1.21)	0.81 (0.59;1.09)	0.17
Total *cis* MUFA[b]				
Mean±SD	10.84±0.93	12.76±0.44	15.16±1.55	
Cases/controls (n)	214/418	207/416	212/414	
OR (95% CI)*	1.00	0.96 (0.75;1.23)	0.94 (0.72;1.22)	0.63
Oleic acid (18:1*n*-9)				
Mean±SD	8.44±0.77	10.08±0.39	12.33±1.49	
Cases/controls (n)	218/417	187/415	228/416	
OR (95% CI)*	1.00	0.85 (0.66;1.09)	0.99 (0.76;1.30)	0.95
Total *trans* ruminant fatty acids[c]				
Mean±SD	0.26±0.06	0.41±0.04	0.62±0.15	
Cases/controls (n)	233/456	173/389	227/403	
OR (95% CI)*	1.00	0.91 (0.67;1.22)	1.14 (0.81;1.61)	0.40
Total *trans* industrial fatty acids[d]				
Mean±SD	0.44±0.06	0.62±0.05	0.98±0.48	
Cases/controls (n)	214/447	199/387	220/414	
OR (95% CI)*	1.00	1.11 (0.83;1.48)	1.15 (0.82;1.64)	0.40
Elaidic acid (18:1*n*-9/12)				
Mean±SD	0.14±0.03	0.24±0.04	0.55±0.19	
Cases/controls (n)	196/419	211/425	226/404	
OR (95% CI)*	1.00	1.12 (0.86;1.47)	1.39 (0.99;1.94)	0.06

Total *cis* *n*-6 PUFA[e]				
Mean±SD	34.08±2.39	37.74±0.64	40.91±1.66	
Cases/controls (n)	214/417	195/415	224/416	
OR (95% CI)*	1.00	0.93 (0.71;1.19)	1.08 (0.84;1.41)	0.49
Linoleic acid (18:2*n*-6)				
Mean±SD	18.72±1.98	22.38±0.79	26.10±1.92	
Cases/controls (n)	197/418	218/414	218/416	
OR (95% CI)*	1.00	1.20 (0.93;1.54)	1.17 (0.90;1.52)	0.23
Long chain *n*-6 PUFA[f]				
Mean±SD	12.40±1.61	15.05±0.55	17.68±1.43	
Cases/controls (n)	221/416	195/418	217/414	
OR (95% CI)*	1.00	0.83 (0.64;1.08)	0.98 (0.74;1.28)	0.85
Total *cis* *n*-3 PUFA[g]				
Mean±SD	5.26±0.68	6.98±0.47	9.92±2.19	
Cases/controls (n)	230/426	216/408	187/414	
OR (95% CI)*	1.00	0.91 (0.71;1.16)	0.78 (0.59;1.04)	0.09
α-linolenic acid (18:3*n*-3ccc)				
Mean±SD	0.12±0.02	0.18±0.02	0.28±0.07	
Cases/controls (n)	226/473	169/365	238/410	
OR (95% CI)*	1.00	1.01 (0.76;1.32)	1.30 (0.98;1.72)	0.06
Long chain *n*-3 PUFA[h]				
Mean±SD	5.06±0.67	6.76±0.48	9.71±2.19	
Cases/controls (n)	222/417	223/415	188/416	
OR (95% CI)*	1.00	0.93 (0.73;1.20)	0.80 (0.61;1.06)	0.13
Total *cis* PUFA[i]				
Mean±SD	42.35±1.74	45.06±0.53	47.38±1.34	
Cases/controls (n)	218/416	208/416	207/416	
OR (95% CI)*	1.00	0.97 (0.75;1.26)	0.95 (0.73;1.24)	0.73
Ratio *n*-6/*n*-3 PUFA				
Mean±SD	3.69±0.78	5.47±0.44	7.78±1.54	
Cases/controls (n)	195/418	216/414	222/416	
OR (95% CI)*	1.00	1.12 (0.87;1.45)	1.21 (0.91;1.60)	0.19

DI_{16} (16:1*n*-7/n-9/16:0)**				
Cases/controls (n)	399/793	180/378	52/77	
OR (95% CI)*	1.00	0.94 (0.74;1.19)	1.22 (0.18;1.84)	0.70
DI_{18} (18:1n-9/18:0)				
Cases/controls (n)	214/421	216/432	203/395	
OR (95% CI)*	1.00	0.99 (0.77;1.28)	0.95 (0.72;1.25)	0.71

 # OR = odd ratio; CI = confidence interval
† P values < 0.05 are shown in boldface
** Cases and controls (1:2) are matched for centre, menopausal status, age, fasting status and time of the day at blood collection, and adjusted for duration of oral contraceptive use, parity, menopausal status, and total energy intake,.*
*** DI =Desaturation Index.*
[a]Total SFA included 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; [b]Total cis MUFA included 14:1, 15:1, 16:1n-7/n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1; [c]Total trans ruminant fatty acids included 18:1n-7t, CLA; [d]Total trans industrial fatty acids included 16:1n-7t/n-9t, 18:1n-12/n-9t, 18:2n-6tt, 18:2n-6tc, 18:3n-3ttt; [e]Total n-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5; [f]Total long-chain n-6 PUFA included 20:2, 20:3, 20:4, 22:4, 22:5; [g]Total n-3 PUFA included 18:3, 18:4, 20:4, 20:5, 22:5, 22:6; [h]Total long-chain n-3 PUFA included 20:4, 20:5, 22:5, 22:6; [i]Total cis PUFA included total n-6 PUFA and total n-3 PUFA.

Figure legends

Figure 1. Association between *n*-6 linoleic acid and EOC risk according to dietary sources.

The percentage of contribution next to the food item was calculated for each food (sub-) group based on the mean daily intake reported in the dietary questionnaire. It represents the contribution of the correspondent food to the linoleic acid intake. HR = Hazard Ratio; CI = confidence interval. The multivariable model was adjusted for duration of oral contraceptive use, parity, menopausal status at enrolment and total energy intake.

Figure 2. Association between *n*-3 α-linolenic acid and EOC risk according to dietary sources.

The percentage of contribution next to the food item was calculated for each food (sub-) group based on the mean daily intake reported in the dietary questionnaire. It represents the contribution of the correspondent food to the alpha-linoleic acid intake. HR = Hazard Ratio; CI = confidence interval. The multivariable model was adjusted for duration of oral contraceptive use, parity, menopausal status at enrolment and total energy intake.