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, which are risk factors for ovarian cancer. Few epidemiologic studies have investigated the association of fatty acids with ovarian cancer.

Methods: Within the European Prospective Investigation into Cancer and Nutrition (EPIC), 1,486 incident ovarian cancer cases were identified. Cox proportional hazard models with adjustment for ovarian cancer risk factors were used to estimate HRs 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 ORs 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 [HR comparing fifth with first quintile$_{Q5\text{-}Q1}$ = 1.29; 95% confidence interval (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 (OR comparing third with first 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).

Conclusions: 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.

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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-related 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; ref. 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 (PUFA) 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 sociodemographic and lifestyle data were collected at enrollment 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 forms were provided by all participants. The study was conducted in accordance with the Declaration of Helsinki and was ethically approved by 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 standardized 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; ref. 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 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; ref. 16). The correlation coefficients between three measures over a 2-year 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 EOCs were identified through population-based cancer registries or active follow-up. EOCs 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 EOCs were identified after a mean follow-up of 8.2 years. Cases were censored if they were nonepithelial ($n = 76$), or tumors of borderline malignancy ($n = 62$), leaving 1,486 EOC cases for the current analysis. Cancer endpoint data is based on the latest round of follow-up received from the EPIC centers and centralized at IARC between 2014 and 2016. For each EPIC study center, 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, menstrual cycle phase for premenopausal women, and 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 saturated fatty acids (SFA) to long-chain PUFA, including 15 *trans*-fatty acid isomers from industrial processes and animal sources, has been described previously (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).

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Table 1. Characteristics of the study population.

EPIC-wide study $N = 323,376$	EOC cases $n = 1,486$	Noncases[a] $n = 321,890$	Nested case–control study	EOC cases $n = 633$	Controls $n = 1,248$	P[b]
Anatomic subtypes, number, (%[c])						
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[c]						
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[c]						
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), %[c]	14.8	12.3		19.9	17.5	0.20
Reproductive and hormone factors						
Number of full-term pregnancies[d]	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, %[c]						<**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[e], %[c]						0.84
Never	67.3	75.2		74.6	74.2	
Ever	32.7	24.8		25.4	25.8	
Ever breastfed[d], %[c]						0.10
No	28.6	27.8		28.4	24.8	
Yes	71.4	72.2		71.6	75.2	
Ovariectomy, %[c]						<**0.01**
No	97.6	95.8		98.4	95.4	
Unilateral	2.4	4.2		1.6	4.6	
Menopausal status, %[c]						Matched
Premenopausal	20.8	36.0		25.7	24.9	
Postmenopausal	61.2	44.4		59.9	59.5	
Perimenopausal	17.9	19.6		14.4	15.5	
Age at menopause[e]	49.6 ± 4.7	48.9 ± 4.8		49.7 ± 4.5	49.1 ± 4.7	0.07
Socioeconomic status and lifestyle						
Total energy intake, kcal/day	1,959.1 ± 527.9	1,991.6 ± 545.4		2,002.1 ± 540.3	1,993.1 ± 514.4	0.73
Alcohol intake, %[c]						**0.01**
None	7.7	6.7		8.8	7.1	
<5 g/day	49.9	48.9		56.8	52.2	
5–<14.9 g/day	26.4	27.3		20.2	25.9	
15.0–<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, %[c]						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	

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Table 1. Characteristics of the study population. (Cont'd)

EPIC-wide study $N = 323,376$	EOC cases $n = 1,486$	Noncases[a] $n = 321,890$	Nested case–control study	EOC cases $n = 633$	Controls $n = 1,248$	P^b
Smoking status, %[c]						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)[c]						
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[f] (g/day or mg/day) Median (95% CI)[c]			**Phospholipid fatty acids[f] (% of total fatty acids) Mean ± SD[c]**			
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 (258.7–2,066.4)	665.4 (237.7–1,907.2)	*n*-3 PUFA	7.3 ± 2.3	7.3 ± 2.3	0.54
α-linolenic acid (mg/day)	421.2 (122.7–1,326.5)	383.1 (117.5–1,252.1)	α-linolenic acid	0.2 ± 0.1	0.2 ± 0.1	0.47
n-3 long-chain PUFA (mg/day)	208.3 (24.5–1,129.2)	196.8 (21.9–1,037.9)	*n*-3 long-chain PUFA	7.1 ± 2.1	7.1 ± 2.3	0.52

[a]Considered as noncases at the most recent cancer endpoint and vital status update.
[b]Student *t* test for continuous variables and χ^2 test for categorical variables in the nested case–control approach.
[c]Continuous variables are presented as means ± SD or median (95% CI). Categorical variables are presented as percentages. Missing values were excluded from percentage calculations.
[d]Among parous women.
[e]Among postmenopausal women only.
[f]Groupings of fatty acids are as described in Materials and Methods.

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 (Lyon, France).

Using values for 60 individual fatty acids, we calculated the percentage of the following groups: SFA, *cis* monounsaturated fatty acid (MUFA), ruminant *trans*-fatty acid (rTFA), industrial *trans*-fatty acid (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

In the descriptive statistics for sociodemographic and lifestyle characteristics and dietary intake of fatty acids, frequencies were reported for the categorical variables and means ± SDs were calculated for the continuous variables. HRs and 95% confidence intervals (CI) for the association between dietary fatty acids and EOC risk were calculated by Cox proportional hazards regression using age as the time metric; the entry time was age at recruitment and the exit time was age at cancer diagnosis, death, emigration, or last complete follow-up, whichever occurred first. Fatty acid intake among all cohort participants was stratified into quintiles, and the lowest category was set as the reference group. All models were stratified by the study center and age at enrollment. The retained multivariable model was adjusted for duration of oral contraceptive use (never use; use <5 years; use ≥5 years; missing), parity (number of live and/or stillborn children: 0; 1–2; 3–4; >4; missing), menopausal status at enrollment (premenopausal; postmenopausal; perimenopausal/unknown menopause), and total energy intake (continuous). Additional potential confounders [history/duration of breastfeeding, ever use of postmenopausal hormones, history of unilateral ovariectomy, body mass index (BMI), physical activity, tobacco smoking, education status, and intake of

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

	Q1 Reference	Q2	Q3	Q4	Q5	P_{trend}[a]	q_{trend}[b]
Total SFA[c]							
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/noncases (*n*)	329/64,342	303/64,368	326/64,344	269/64,402	259/64,411		
HR (95% CI)[d]	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/noncases (*n*)	341/64,331	299/64,371	329/64,341	250/64,421	267/64,403		
HR (95% CI)[d]	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 SFA[e]							
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/noncases (*n*)	334/64,337	309/64,363	267/64,403	301/64,369	275/64,395		
HR (95% CI)[d]	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[f]							
Mean intake ± SD (g/d)	13.76 ± 2.63	19.77 ± 1.39	24.65 ± 1.47	30.64 ± 2.11	44.32 ± 9.44		
Cases/noncases (*n*)	346/64,325	326/64,345	295/64,375	285/64,386	234/64,436		
HR (95% CI)[d]	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/noncases (*n*)	342/64,329	341/64,331	286/64,383	282/64,389	235/64,435		
HR (95% CI)[d]	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 rTFA[g]							
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/noncases (*n*)	382/64,289	293/64,381	277/64,390	291/64,382	243/64,425		
HR (95% CI)[d]	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 iTFA[h]							
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/noncases (n)	234/64,437	255/64,416	286/64,384	323/64,348	388/64,282		
HR (95% CI)[d]	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/noncases (*n*)	238/64,433	254/64,417	280/64,390	323/64,348	391/64,279		
HR (95% CI)[d]	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[i]							
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/noncases (*n*)	308/64,363	271/64,400	283/64,387	327/64,344	297/64,373		
HR (95% CI)[d]	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/noncases (*n*)	309/64,362	270/64,401	280/64,390	329/64,342	298/64,372		
HR (95% CI)[d]	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[j]							
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/noncases (*n*)	287/64,384	337/64,348	278/64,384	288/64,377	296/64,374		
HR (95% CI)[d]	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[k]							
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/noncases (*n*)	252/64,420	273/64,397	281/64,389	308/64,363	372/64,298		
HR (95% CI)[d]	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/noncases (*n*)	260/64,411	262/64,409	278/64,393	306/64,364	380/64,290		
HR (95% CI)[d]	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[l]							
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/noncases (*n*)	273/64,398	293/64,378	276/64,395	300/64,370	344/64,326		
HR (95% CI)[d]	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[m]							
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/noncases (*n*)	300/64,371	268/64,403	278/64,392	330/64,341	310/64,360		
HR (95% CI)[d]	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**

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Table 2. Association of estimated dietary intakes of fatty acids with ovarian cancer risk in the EPIC cohort. (Cont'd)

	Q1 Reference	Q2	Q3	Q4	Q5	P_{trend}[a]	q_{trend}[b]
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/noncases (*n*)	405/64,266	299/64,372	262/64,408	279/64,392	241/64,429		
HR (95% CI)[d]	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

[a]*P* or *q* values < 0.05 are shown in boldface type.
[b]Value for FDR correction.
[c]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.
[d]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
[e]Odd chain fatty acids included 15:0, 17:0.
[f]Total *cis* MUFA included 16:1*n*-7/*n*-9, 17:1, 18:1*n*-5, 18:1*n*-7, 18:1*n*-9, 20:1, 22:1, 24:1.
[g]Total rTFA included 18:1*n*-7t, CLA.
[h]Total iTFA included 16:1*n*-9t, 18:1*n*-9t, 18:2*n*-6tt, 18:3*n*-3ttt.
[i]Total *n*-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4.
[j]Total long-chain *n*-6 PUFA included 20:2, 20:3, 20:4.
[k]Total *n*-3 PUFA included 18:3, 20:3, 20:5, 22:5, 22:6.
[l]Total long-chain *n*-3 PUFA included 20:3, 20:5, 22:5, 22:6.
[m]Total *cis*-PUFA included total *n*-6 PUFA and total *n*-3 PUFA.

alcohol, red meat, or total sugar] did not alter relative risks by 10% or more so were not included in the final models. A similar effect was observed for the mutual adjustment of fatty acids for one another. For each fatty acid, quintile-specific medians were used to compute the trend tests.

Q-values were calculated using the false discovery rate of the Benjamini–Hochberg procedure to correct for multiple testing (20).

In addition, the associations between dietary sources of individual fatty acids (as continuous variables) and EOC risk were investigated. The selected dietary sources were those that contributed to more than 1% of fatty acid 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 RR (or HR) in the current cancer cases:

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

With RR_i and p_i expressing the adjusted HR 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, HRs were correct approximations of risk ratios (RR_i). Confidence intervals were calculated by bootstrap sampling (22).

Plasma phospholipid fatty acid values were log-transformed, and geometric means with 95% CI were reported. Fatty acid values were divided into tertiles based on the distribution among the controls, and conditional logistic regression was used to estimate the effect on EOC risk. Models were adjusted for the same confounders as those selected above for the analyses on dietary intakes.

Cox proportional hazards competing risk analysis (23) was used to estimate HR and 95% CI by menopausal status. Heterogeneity tests 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 analyses were carried out using STATA 14.0 (StataCorp). *P* values below 0.05 were considered statistically significant.

Results

Compared with the noncases, the EOC cases were more likely to have a higher BMI, be nulliparous, be postmenopausal, have ever used HRT, and 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; P_{trend} = 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 third with first 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 $\rho = 0.88$, $P < 0.001$). 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

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

Plasma phospholipid fatty acids (% of total fatty acids)	Tertile 1 Reference	Tertile 2 OR (95% CI)	Tertile 3 OR (95% CI)	P_{trend}[a]
Total SFA[b]				
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)[c]	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)[c]	1.00	0.94 (0.73–1.21)	0.81 (0.59–1.09)	0.17
Total *cis* MUFA[d]				
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)[c]	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)[c]	1.00	0.85 (0.66–1.09)	0.99 (0.76–1.30)	0.95
Total rTFA[e]				
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)[c]	1.00	0.91 (0.67–1.22)	1.14 (0.81–1.61)	0.40
Total iTFA[f]				
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)[c]	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)[c]	1.00	1.12 (0.86–1.47)	1.39 (0.99–1.94)	0.06
Total *cis n*-6 PUFA[g]				
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)[c]	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)[c]	1.00	1.20 (0.93–1.54)	1.17 (0.90–1.52)	0.23
Long-chain *n*-6 PUFA[h]				
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)[c]	1.00	0.83 (0.64–1.08)	0.98 (0.74–1.28)	0.85
Total *cis n*-3 PUFA[i]				
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)[c]	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)[c]	1.00	1.01 (0.76–1.32)	1.30 (0.98–1.72)	0.06
Long chain *n*-3 PUFA[j]				
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)[c]	1.00	0.93 (0.73–1.20)	0.80 (0.61–1.06)	0.13
Total *cis* PUFA[k]				
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)[c]	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)[c]	1.00	1.12 (0.87–1.45)	1.21 (0.91–1.60)	0.19

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Table 3. Association of plasma phospholipid fatty acids with ovarian cancer risk in the EPIC cohort. (Cont'd)

Plasma phospholipid fatty acids (% of total fatty acids)	Tertile 1 Reference	Tertile 2 OR (95% CI)	Tertile 3 OR (95% CI)	P_{trend}[a]
DI_{16} (16:1*n*-7/n-9/16:0)				
Cases/controls (*n*)	399/793	180/378	52/77	
OR (95% CI)[c]	1.00	0.94 (0.74–1.19)	1.22 (0.18–1.84)	0.70
DI_{18} (18:1*n*-9/18:0)				
Cases/controls (*n*)	214/421	216/432	203/395	
OR (95% CI)[c]	1.00	0.99 (0.77–1.28)	0.95 (0.72–1.25)	0.71

Abbreviation: DI, desaturation index.
[a]*P* values < 0.05 are shown in boldface.
[b]Total SFA included 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0.
[c]Cases and controls (1:2) are matched for study center, 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.
[d]Total *cis* MUFA included 14:1, 15:1, 16:1*n*-7/*n*-9, 17:1, 18:1*n*-5, 18:1*n*-7, 18:1*n*-9, 20:1, 22:1, 24:1.
[e]Total rTFA included 18:1*n*-7t, CLA.
[f]Total iTFA included 16:1*n*-7t/*n*-9t, 18:1*n*-12/*n*-9t, 18:2*n*-6tt, 18:2*n*-6tc, 18:3*n*-3ttt.
[g]Total *n*-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5.
[h]Total long-chain *n*-6 PUFA included 20:2, 20:3, 20:4, 22:4, 22:5.
[i]Total *n*-3 PUFA included 18:3, 18:4, 20:4, 20:5, 22:5, 22:6.
[j]Total long-chain *n*-3 PUFA included 20:4, 20:5, 22:5, 22:6.
[k]Total *cis* PUFA included total *n*-6 PUFA and total *n*-3 PUFA.

($HR_{Q5\text{-}Q1}$ = 1.10; 95% CI, 1.01–1.21; **Fig. 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; **Fig. 1**). The overall positive association between α-linolenic acid and EOC risk was mainly driven by the contribution of deep-frying fat ($HR_{Q5\text{-}Q1}$ = 1.18; 95% CI = 1.05–1.34) and margarine ($HR_{Q5\text{-}Q1}$ = 1.02; 95% CI = 1.01–1.04; **Fig. 2**).

Stratified analysis by menopausal status showed a positive association between palmitic acid and EOC risk restricted to premenopausal women ($HR_{Q5\text{-}Q1}$ = 2.13; 95% CI = 1.22–3.71),

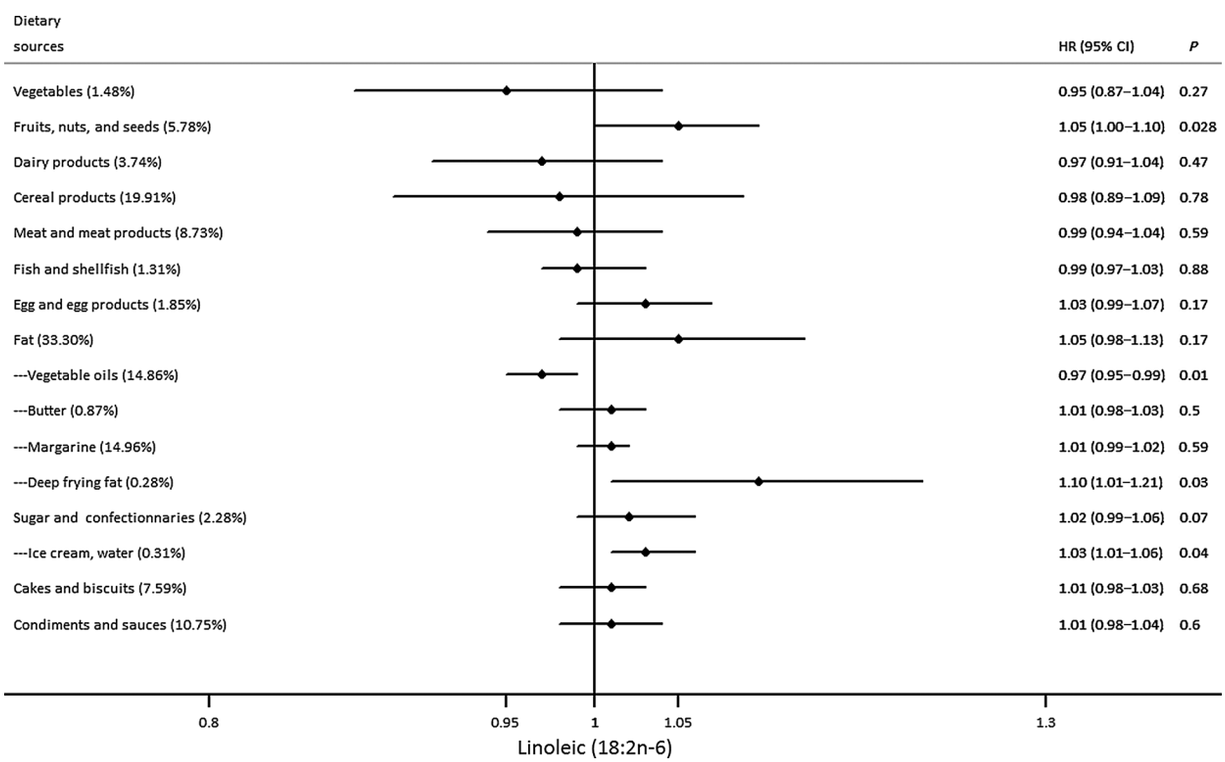

Figure 1.
Association between *n*-6 linoleic acid and EOC risk according to dietary sources. The percentage of contribution next to the food item was calculated for each food (sub-) group based on the mean daily intake reported in the dietary questionnaire. It represents the contribution of the correspondent food to the linoleic acid intake. The multivariable model was adjusted for duration of oral contraceptive use, parity, menopausal status at enrollment, and total energy intake.

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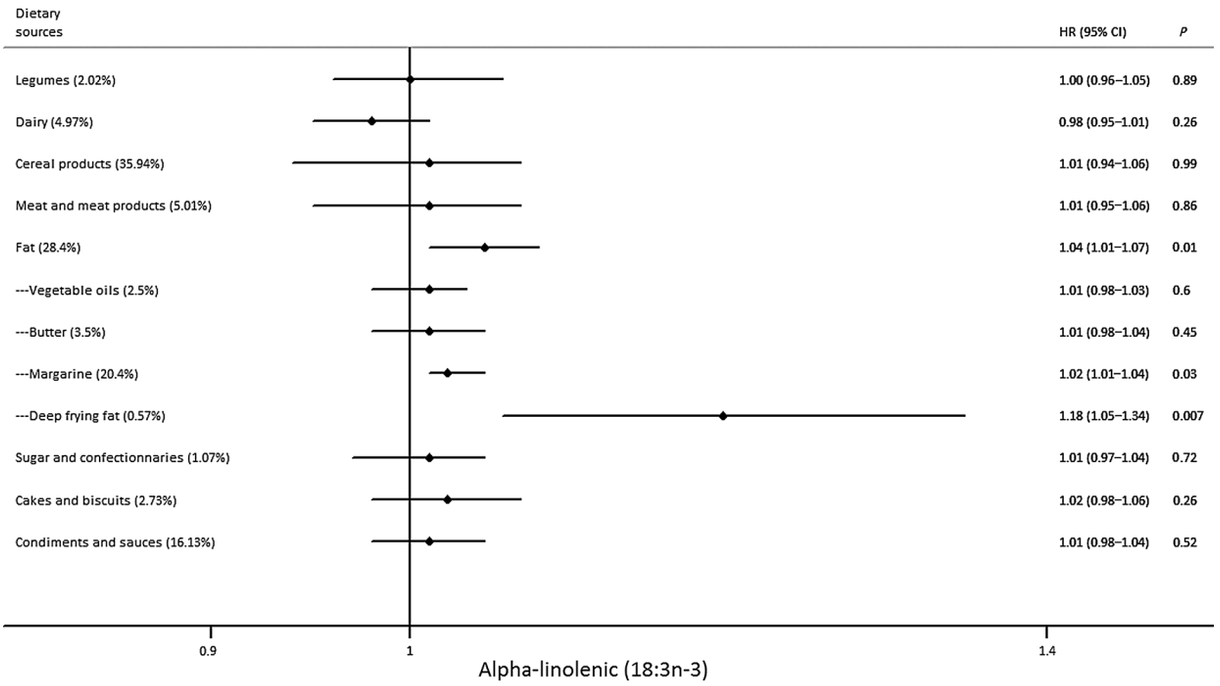

Figure 2.

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 α-linoleic acid intake. The multivariable model was adjusted for duration of oral contraceptive use, parity, menopausal status at enrollment, and total energy intake.

while no association was found in postmenopausal women ($P_{\text{heterogeneity}} = 0.04$). All $P_{\text{heterogeneity}} > 0.05$.

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 histologic 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, 28–407 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 this 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 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. 1,486 in this 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 α-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 PUFA compared with the limited conversion of α-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 egg products and total fat were reported, but not significant. In

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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 coexposure 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. In addition, 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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