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Dietary and circulating fatty acids in association with ovarian and endometrial cancers

Sahar Ghantous

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Epidémiologie, santé publique, recherche sur les services de santé
Discipline :
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Sahar YAMMINE

**Dietary and circulating fatty acids in
association with ovarian and
endometrial cancers**

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N°d'ordre NNT : xxx

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Résumé

Peu de facteurs de risque modifiables ont été identifiés pour les cancers de l'ovaire (CO) et de l'endomètre (CE). L'identification de ces facteurs et la mise en place de mesures préventives sont donc nécessaires. Cette thèse vise à mieux comprendre l'étiologie de ces deux cancers, en mettant l'accent sur le rôle de la nutrition en particulier les acides gras (AG).

Dans la cohorte Européenne EPIC, nous avons évalué l'association entre les apports alimentaires estimés, les mesures plasmatiques des AG et le risque du CO. Ces deux approches ont montré que les apports alimentaires et les niveaux plasmatiques de l'acide *trans*-élaïdique d'origine industrielle étaient associés à un risque élevé du CO. De plus, une association positive a été détectée avec des apports élevés en acides linoléique et α -linoléique provenant des aliments frits.

Nous avons également mené une étude EPIC entre les apports alimentaires d'AG et le risque du CE. Des associations négatives ont été trouvées entre les sources végétales de l'acide γ -linoléique et celles de l'acide α -linoléique et le risque du CE.

De telles études seraient importantes dans les pays en développement ; pour cela nous avons évalué, dans une étude pilote menée dans une cohorte Libanaise, les associations entre les AG sériques et des indicateurs de l'obésité, un facteur de risque pour le CO et la CE.

Nos données suggèrent donc que l'élimination des AG *trans* industriels pourrait réduire le risque du CO. Le risque du CE pourrait également diminuer en adhérant à une alimentation riche en végétaux. Notre étude au Liban pourrait être une base pour des travaux futurs visant à étudier les associations entre la nutrition et le risque du cancer.

Mots-clés : acides gras, cancer de l'ovaire, cancer de l'endomètre, obésité, épidémiologie, questionnaires, biomarqueurs.

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Abstract

As the incidence of ovarian cancer (OC) and endometrial cancer (EC) is rising worldwide, prevention strategies are needed. However, few preventable factors have been identified. Thus, their identification and implementation are warranted. This thesis aimed to better understand the the role of nutrition particularly fatty acids (FA).

Within the European EPIC cohort including ~ 300 000 women, we assessed the association between estimated dietary intakes of FA, circulating FA and OC risk. Both approaches convened that higher dietary intakes and circulating levels of industrial *trans* FA were associated with greater OC risk. A positive association was also detected for higher intakes of linoleic acid and α -linolenic acid originating mainly from deep frying fat.

We conducted an EPIC study between dietary intakes of FA and EC risk where we found negative associations with γ -linolenic acid and α -linolenic acid intakes from plant sources.

Such studies would be relevant in low-middle-income countries (LMICs), undergoing nutrition transition, characterized by the adoption of westernized diets. In this context, we assessed in a recent Lebanese cohort, the associations between serum FA and obesity, a risk factor for OC and EC. In this population, high markers of endogenous FA synthesis were positively correlated with adiposity.

Our data suggest that eliminating industrial *trans* FA intake could reduce OC risk. The risk of EC may be decreased by adhering to a diet high in vegetables. Our study conducted in Lebanon could provide a baseline for future work aiming to study dietary factors and cancer risk in LMICs.

Keywords: fatty acids, ovarian cancer, endometrial cancer, obesity, epidemiology, questionnaires, biomarkers.

Institute hosting the thesis candidate:

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Résumé substantiel

En 2018, le cancer de l’ovaire (CO) a été classé le septième cancer le plus fréquent chez la femme au niveau mondial et le cancer de l’endomètre (CE) sixième.

L’étiologie de ces deux cancers a été largement explorée. Bien que les facteurs non-évitablement soient bien identifiés, ils n'expliquent qu'une faible proportion des nouveaux cas de CO et CE. En outre, des études épidémiologiques ont permis d'identifier un nombre limité de facteurs de risque modifiables. Cependant, les évaluations du rôle de la nutrition, particulièrement des acides gras (AG), sont encore limitées et peu concluantes, avec un faible potentiel à ce jour pour la mise en œuvre des politiques de prévention primaire.

Le but de cette thèse est donc d’évaluer le rôle des AG dans l’étiologie de ces deux cancers dans l’étude prospective européenne sur le cancer et la nutrition (EPIC), une cohorte multicentrique de ~ 300 000 femmes provenant de 10 pays européens.

En premier lieu, nous avons évalué l'association entre les apports alimentaires estimés et les mesures plasmatiques des AG, et le risque du CO. Au moment de l’étude, 1 486 cas incidents du CO ont été identifiés. Des modèles de Cox à risques proportionnels avec l’âge comme échelle de temps ont permis d’estimer des risques relatifs (Hazard ratios (HR)) et leur intervalle de confiance à 95% (IC95%). Ces modèles ont été ajustés pour des facteurs de risque du CO et utilisés pour estimer le risque de CO selon les quintiles de consommation d'AG. Un « False Discovery Rate (FDR) » a été calculé pour contrôler l’usage de tests multiples. Des modèles de régression logistique conditionnelle multivariable ont été utilisés pour estimer le risque du CO parmi les tertiles d'AG plasmatiques pour 633 cas appariés à deux témoins dans une étude cas-témoins nichée à EPIC. Une association positive a été trouvée entre le risque du CO et l’apport alimentaire de l’acide *trans*-élaïdique d’origine industrielle (Hazard Ratio

comparant 5^{ème} et 1^{er} quintile $Q_5-Q_1=1.29$; $IC_{95\%} = 1.03-1.62$; $ptrend=0.02$, $q-value=0.06$). Les apports alimentaires élevés des acides n-6 linoléique ($HR = 1.10$; $IC_{95\%}=1.01-1.21$; $ptrend=0.03$) et n-3 α -linoléique ($HR = 1.18$; $IC_{95\%} = 1.05-1.34$; $ptrend=0.007$) provenant principalement de l'huile de friture étaient également associés à une augmentation du risque du CO. En assumant que l'association est causale, l'estimation de la fraction attribuée à la population (PAF) a indiqué que 11.7% ($IC_{95\%} (1.9\%, 27.4\%)$) du risque du CO peuvent être attribués à l'acide *trans* élaïdique.

Des tendances positives ont été aussi trouvées entre le risque du CO et les acides *trans* élaïdique plasmatique (Odds Ratio comparant 3^{ème} with 1^{er} tertile $T_3-T_1 = 1.39$; $IC_{95\%}=0.99-1.94$; $ptrend=0.06$) et α -linoléique ($OR_{T_3-T_1}=1.30$; $IC_{95\%}=0.98-1.72$; $ptrend=0.06$).

Ces deux approches ont montré que les apports alimentaires et les niveaux plasmatiques de l'acide *trans*-élaïdique industriel étaient associés à un risque élevé du CO. De plus, une association positive a été détectée avec des apports élevés en acides linoléique et α -linoléique provenant principalement des aliments frits.

Nous avons également mené une étude EPIC entre les apports alimentaires d'AG et le risque du CE. Au moment de l'étude, 1 886 cas incidents du CE ont été identifiés. Des modèles de régression de Cox ajustés pour des facteurs de risque du CE ont été utilisés pour estimer le risque du CE selon les quintiles de consommation d'AG. FDR a été calculé pour contrôler l'usage de tests multiples. Une association négative a été trouvée entre l'apport de l'acide γ -linoléique ($HR_{Q_5-Q_1}=0.77$, $IC_{95\%} = 0.64 ; 0.92$, $ptrend=0.01$, $q-value=0.15$) probablement due à ses sources végétales ($HR=0.94$, $IC_{95\%} = (0.90-0.98)$, $p=0.01$) et non animales ($HR= 1.02$, $IC_{95\%} = (0.94; 1.10)$, $p=0.62$). De plus, des associations négatives similaires ont été trouvées entre les sources végétales de l'acide n-3 α -linoléique et le risque du CE ($HR= 0.93$, $IC_{95\%} = (0.87 ; 0.99)$, $p=0.04$) et non animales ($HR= 1.02$, $IC_{95\%} = (0.95; 1.10)$, $p=0.64$).

Tous les pays sont confrontés aujourd'hui à d'importants défis dans la mise en œuvre de stratégies de prévention contre le fardeau croissant du cancer dans le monde qui menace les économies et appauvrit les individus et les familles, en particulier dans les pays à revenus intermédiaires ou faibles. Étant donné que les aliments industriels représentent des ressources alimentaires relativement peu coûteuses (bien que plus coûteuses pour la santé), l'amélioration de la qualité de l'alimentation peut être une stratégie de prévention du cancer importante particulièrement dans les pays en développement. Pour ces raisons, nous avons évalué, dans une étude pilote menée dans une cohorte Libanaise, les associations entre les AG sériques et des indicateurs de l'obésité, un facteur de risque pour le CO et le CE. Dans cette étude, la composition en AG de 395 échantillons sériques (129 hommes, 266 femmes) a été analysée. Des corrélations de Spearman, ajustées et corrigées pour les erreurs de tests multiples, ont été calculées entre les AG sériques, les indices de désaturation (DI_{16} et DI_{18} sont les indicateurs de la synthèse endogène de l'acide palmitoléique à l'acide palmitique et de l'acide oléique à l'acide stéarique respectivement) et les indicateurs de l'obésité (Indice de Masse Corporelle (IMC) et tour de taille). L'IMC a été positivement corrélé avec les AG saturés chez les hommes ($r = 0.40$, $p < 0.0001$, $q < 0.0001$) et les femmes ($r = 0.33$, $p < 0.0001$, $q < 0.0001$). L'IMC a été aussi positivement corrélé avec l'acide monoinsaturé palmitoléique uniquement chez les femmes ($r = 0.15$, $p = 0.01$, $q = 0.03$). Cette étude a suggéré que les AG saturés et l'acide monoinsaturé palmitoléique probablement dérivé de l'apport alimentaire en AG saturés et de la synthèse endogène, peuvent être associés avec l'obésité dans cette population libanaise. La causalité de ces associations doit être explorée dans des études expérimentales.

Les données de cette thèse suggèrent que l'élimination des AG *trans* d'origine industrielle et des aliments frits pourraient potentiellement réduire le risque de CO. Le risque d'EC peut également être diminué en suivant les recommandations du WCRF sur le respect d'un régime alimentaire riche légumes. Notre étude au Liban (pays en voie de développement)

pourrait fournir une base pour des travaux futurs visant à étudier le risque du cancer en relation avec l'alimentation ainsi que la mise en œuvre de recommandations de santé publique qui seraient opportunes au cours des transitions nutritionnelles

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List of figures and tables

Figures

Figure 1. Different ovarian tumors originate from different cell subtypes. Prevalence of malignant components in parentheses

Figure 2. Origin and histological subtypes associated with type I and type II molecular classification

Figure 3. Age standardized ovarian cancer incidence rates in the world in 2018

Figure 4. Age standardized cancers for women in developed vs developing countries in 2018

Figure 5. World Cancer Research Fund summary on ovarian cancer

Figure 6. Overview of endometrial cancer origin and development

Figure 7. Age standardized endometrial cancer incidence rates in the world in 2018

Figure 8. World Cancer Research Fund summary on ovarian cancer

Figure 9. Fatty Acids: Structures and configurations

Figure 10. Fatty acids: origins and functions

Figure 11. N-6 and N-3 Polyunsaturated fatty acids pathways and roles

Figure 12. Fatty acids and cancer development: Biological plausibility

Figure 13. Quantification of circulating fatty acids

Figure 14. Associations between plant and animal sources of gamma-linolenic acid and endometrial cancer risk

Figure 15. Associations between plant and animal sources of alpha-linolenic acid and endometrial cancer risk

Tables

Table 1. Groupings of fatty acids

Table 2. Number of EPIC subjects by country with questionnaires information and availability of blood samples

Table 3. Characteristics of the study population

Table 4. Association of estimated dietary intakes of fatty acids with endometrial cancer risk in the EPIC cohort

List of abbreviations

ACC: Acetyl-CoA carboxylase

AG: Acide Gras

ASR: Age Standardized Rate

ATP: Adenosine triphosphate

AUB: American University of Beirut

BMI: Body Mass Index

BPA: Bisphenol A

CE: Cancer de l'endomètre

CEBP: Cancer Epidemiology Biomarkers and Prevention

CHD: Coronary Heart Disease

CO: Cancer de l'Ovaire

CRP: C-reactive protein

CVD: Cardio-Vascular Disease

DHA: Docosahexanoic Acid

DI: Desaturation Index

EC: Endometrial Cancer

ECAC: European Code Against Cancer

ENDB: EPIC Nutrient Database

EOC: Epithelial Ovarian Cancer

EPA: Eicosapentaenoic Acid

EPIC: European Prospective Investigation into Cancer

FAS: Fatty Acid Synthase

FFQ: Food Frequency Questionnaires

FID: Flame Ionization Detector

HRT: Hormonal Replacement Therapy

IARC: International Agency for Research on Cancer

IGF: Insulin Growth Factor

IL6: Interleukin 6

iTFA: industrial *Trans* Fatty Acid

LC-PUFA: Long-chain Polyunsaturated Fatty Acid

MUFA: Monounsaturated Fatty Acid

NCD: Non-Communicable Disease

NHS: Nurse's Health Study

NNDSR: National Nutrient Database for Standard Reference of the United States

OC: Ovarian Cancer

PL-FA: Plasma Phospholipid Fatty Acids

PUFA: Polyunsaturated Fatty Acid

rTFA: ruminant *Trans* Fatty Acid

SCD: Stearoyl-CoA desaturase

SFA: Saturated Fatty Acid

US: United States

USDA: United States Databases

WHI: Women Health Initiative

WHO: World Health Organization

Table of Contents

Introduction.....	24
Chapter1.	
Epidemiology of ovarian cancer.....	29
1.1. Ovarian Cancer development	30
1.2. Epidemiological trends of ovarian cancer	32
1.3. Etiology of ovarian cancer.....	34
1.3.1. Non preventable conditions	35
1.3.2. Preventable conditions : Lifestyle and environmental factors	37
Chapter2.	
Epidemiology of endometrial cancer.....	42
2.1. Endometrial cancer development	43
2.2. Epidemiological trends of endometrial cancer	44
2.3. Etiology of endometrial cancer.....	46
2.3.1. Non-preventable conditions.....	46
2.3.2. Preventable conditions: lifestyle and environmental factors.....	48
Chapter 3.	
Fatty Acids, Structures and functions	54
3.1. Structures and functions of fatty acids.....	55
3.2. Fatty Acids and ovarian and endometrial cancers : epidemiological studies.....	61
3.2.1. Fatty acids and ovarian cancer	64
3.2.2. Fatty acids and endometrial cancer.....	65
Chapter4.	
Dietary and circulating fatty acids and ovarian cancer risk in EPIC	68
4.1. Background & aims	69
4.2. Materials & Methods	69
4.2.1. Population study, the EPIC cohort.....	69
4.2.2. Study designs & statistical analyses.....	74
4.3. Results	75
4.4. Conclusion.....	76

4.5. Scientific article.....	77
Chapter 5.	
<i>Dietary fatty acid and endometrial cancer risk within EPIC</i>	122
<i>Abstract</i>	124
5.1. <i>Introduction</i>	125
5.2. <i>Materials & Methods</i>	127
5.3. <i>Results</i>	129
5.4. <i>Discussion</i>	130
5.5. <i>Conclusion</i>	132
5.6. <i>Funding</i>	133
5.7. <i>Tables and figures</i>	134
5.8. <i>References</i>	145
Chapter 6. 149	
<i>Association between Serum Phospholipid Fatty Acid Levels and Adiposity among Lebanese Adults: A Cross-Sectional Study</i>	149
6.1. <i>Background & aims</i>	151
6.2. <i>Materials & Methods</i>	151
6.2.1. <i>Population study</i>	151
6.2.2. <i>Statistical analysis</i>	152
6.3. <i>Results</i>	153
6.4. <i>Discussion</i>	155
6.5. <i>Conclusion and perspectives</i>	156
6.6. <i>Scientific article</i>	156
Chapter 7.	
<i>General conclusion</i>	157
7.1. <i>Main findings and overall discussion</i>	157
7.2. <i>Final conclusion & Public health messages</i>	157

Introduction

With cancer being a major worldwide burden to date, the most important legacy of its epidemiology is the recognition that an array of exogenous exposures is responsible for preventable cancer occurrence. Evidence supporting this statement includes: 1) the notable variation in cancer incidence internationally, 2) migrants' and/or their descendants' frequent development of cancer rates characteristic of the new area of residence, and 3) etiologic studies demonstrating a substantial fraction of cancer arising from exogenous exposures. Among these exposures, the most important factors recognized by Doll and Peto's review in 1981 were nutritional factors (estimated to be responsible for approximately 30-35% (with a range of 10-70%) of cancer occurrence in western populations, today the contribution of nutrition to cancer is estimated to 5.6%) and tobacco use (30 percent, today it is 20%); followed by reproductive factors/sexual behavior; occupation; alcohol drinking; geophysical factors, including ionizing radiation and ultraviolet radiation from sunlight; pollution; iatrogenic exposures and other unknown factors.

Ever since the comprehensive review mentioned above, the field of nutritional epidemiology tried to investigate nutritional exposures and their link with individual cancer sites. Today, diet and nutrition are established as important modifiable risk factors for a substantial proportion of cancers, making this field a great public health target for prevention.

In this context, I have focused my PhD research on the link between nutrition and ovarian and endometrial cancers in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, for which few modifiable risk factors are known.

Among the nutritional factors that might be associated with ovarian and endometrial cancers, I have focused my work on fatty acids. Fatty acids have been shown to have inhibitory or stimulatory effects, according to their type, on cancer growth in different experimental models of carcinogenesis.

In addition, fatty acids are known to impact obesity, and inflammation in experimental studies, both important risk factors for ovarian cancer and endometrial cancer. However, epidemiological results in this field were heterogeneous between prospective and retrospective studies, large and small number of cancer cases, measurements of fatty acids through questionnaires (with potential measurement errors) or blood data; the biomarkers: serum or plasma phospholipid fatty acids representing the past dietary intakes (weeks to months) of fatty acids particularly for those that cannot be endogenously synthesized.

The EPIC is a large-scale prospective study with a multicenter setting (23 centers from 10 European countries), combining study populations with different dietary habits, lifestyles, cancer incidences, dietary questionnaires and blood samples, targeting to increase the overall statistical power, providing a larger variability of dietary exposures and cancer outcomes, and limiting biases.

In this context and with the complete picture of fatty acids assessments that EPIC provided, the overall aims of my thesis were:

- 1- Determine the associations between dietary (estimated through food frequency questionnaires (FFQ)) and plasma phospholipid fatty acids (biomarkers measured through gas chromatography) and ovarian and endometrial cancer risks, overall and by different levels of stratifications.
- 2- Determine the dietary sources of fatty acids and their link with ovarian and endometrial cancer risks.

In **Chapters 1 and 2** of this work, I have detailed the epidemiological, physio-pathological and etiological (including preventable and non-preventable conditions) aspects of ovarian and endometrial cancers. Then in **Chapter 3**, I have reviewed the biochemistry of fatty acids and the epidemiological literature findings on the associations between fatty acids, ovarian and endometrial cancers.

Chapter 4 is the presentation of the first study on fatty acids and ovarian cancer risk, published in Cancer Epidemiology Biomarkers and Prevention (CEBP).

Chapter 5 is dedicated to the study of the association between estimated fatty acids and endometrial cancer risk. This work is ongoing and will be submitted soon.

In addition to this work undertaken within the EPIC cohort, I had the opportunity to collaborate with the American University of Beirut (AUB) on an ongoing cohort aimed originally to examine exposure to bisphenol A (BPA) among adults (>18 years) residing in Beirut. Knowing that fatty acids have been linked with metabolic syndromes in epidemiological and experimental studies, I presented, in **Chapter 6**, a published study on the associations between serum phospholipid fatty acids and obesity indicators in a pilot study in Lebanon, a Mediterranean country where the rates of obesity are escalating.

To conclude, **Chapter 7** ensues with a general discussion on the findings and topics that were touched upon throughout this thesis.

Chapter 1. Epidemiology of ovarian cancer

1.1. Ovarian Cancer development

The normal ovary develops from the gonadal ridge and contains three major cell types: 1) germ cells that are derived from the endoderm and that migrate to the gonadal ridge where they proliferate and differentiate into oocytes, 2) endocrine and interstitial cells that produce estrogen and progesterone, and 3) epithelial cells that are derived from the Mullerian duct and that cover the ovary and line inclusion cysts immediately below the ovarian surface (1). Ovarian cancer (OC) can arise from any of these cell types. The majority of OC are sporadic and arise from an accumulation of genetic damage (2). Both benign and malignant tumors are classified according to the World Health Organization (WHO) into three major groups, namely epithelial (the most frequent), sex cord and ovarian stroma, and germ cell tumors (3) (**Figure 1**). In addition to benign and malignant epithelial lesions, borderline tumors of low-malignant potential contain morphologically and molecularly partially transformed epithelial cells that do not invade underlying stroma. Approximately 10% of borderline tumors can recur after resection and prove lethal (1). According to the criteria proposed by the WHO in 2014, Epithelial Ovarian Cancer (EOC) can be divided into seven histological subcategories; the most common are namely serous, mucinous, endometrioid, clear cell, and undifferentiated (3). Clinical observations and genetic studies have divided OC into two major subtypes (**Figure 2**): Types I EOC are low grade of serous, mucinous, endometrioid, or clear-cell cancers. They are often diagnosed in an early stage (I or II), grow locally and metastasize late. The most prevalent types II EOC are high grade of serous, endometrioid, or undifferentiated histotype. These are highly aggressive and present at late stage (III–IV) (1, 4, 5).

Figure 1. Different ovarian tumors originate from different cell subtypes. Prevalence of malignant components in parentheses (6).

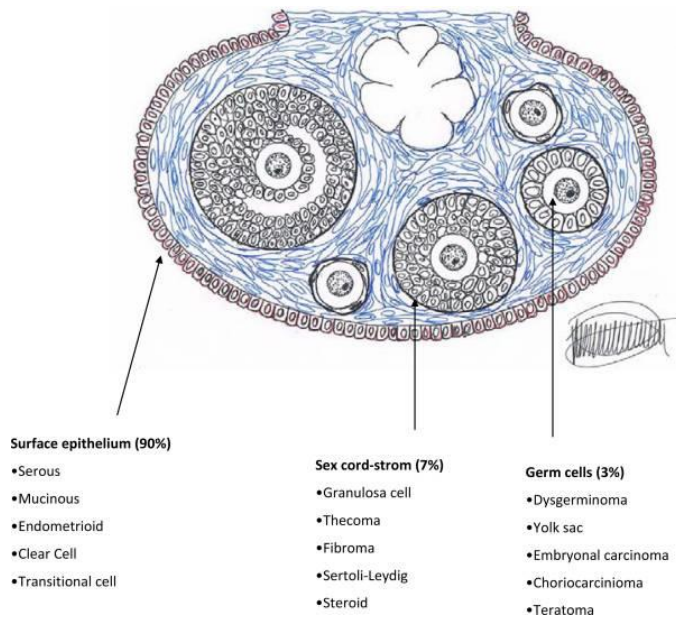
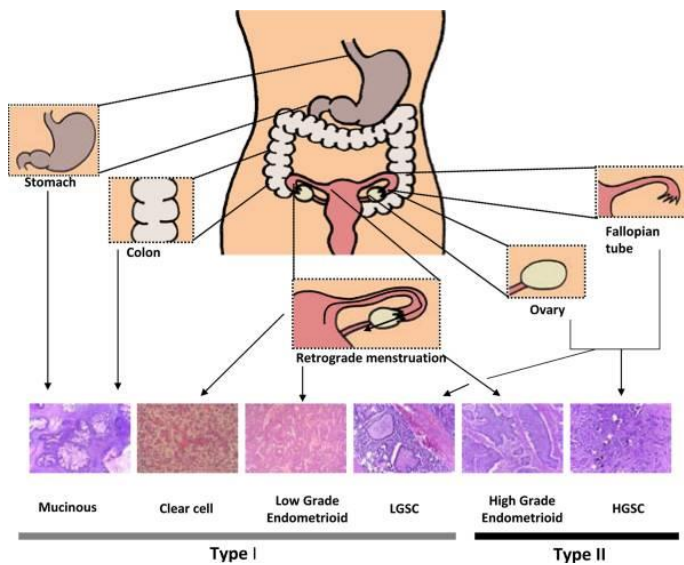


Figure 2. Origin and histological subtypes associated with type I and type II molecular classification (7).

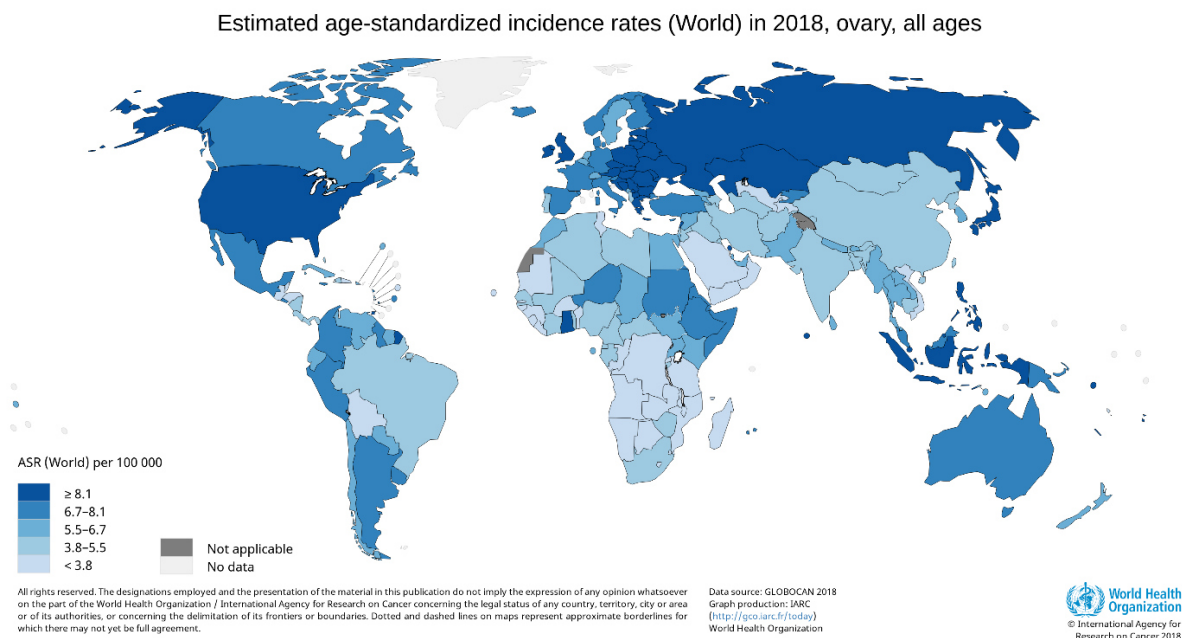


1.2. Epidemiological trends of ovarian cancer

OC is one of the most common gynecologic cancers that rank third after cervical and uterine cancer with the worst prognosis and the highest mortality rate in the world (8). In 2018, OC ranked the seventh most common cancer among women (**Figure 3**) with 295,414 new cases (accounting for 3.4% of all cancer cases in women) and 184,799 deaths (Accounting for 4.4% of the entire cancer-related mortality among women) worldwide (9). Although OC has a lower prevalence in comparison to breast cancer, it is three times more lethal (10), and it is predicted that, by the year 2040, the mortality rate of this cancer will rise significantly (9). It is a silent killer cancer for which the mortality rate is caused by asymptomatic and secret growth of the tumor, delayed onset of symptoms, and lack of proper screening in the world that result in its diagnosis in the advanced stages (11).

The Age Standardized Rate (ASR) of OC is estimated to be 6.6 in 2018 worldwide (9). The incidence of EOC varies in different age and race groups (12). Its incidence is also higher among transitioned countries (9), and approximately 30% of OC cases occur in European countries (11) (**Figure 4**). The highest age-adjusted incidence rates are observed in developed parts of the world, including North America and Central and Eastern Europe, with rates generally exceeding 8 per 100,000. Rates are intermediate in South America (5.8 per 100,000), and lowest in Asia and Africa (≤ 3 per 100,000). Migration from countries with low rates to those with high rates results in greater risk (13).

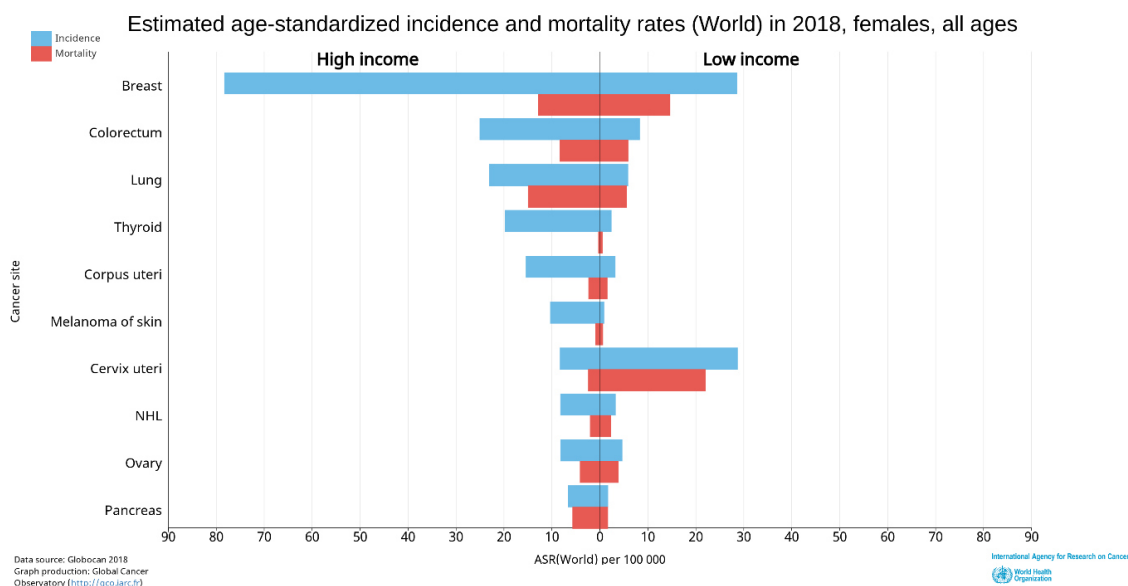
Figure 3. Age standardized ovarian cancer incidence rates in the world in 2018



Source: <http://globocan.iarc.fr>

Figure 4. Age standardized cancers for women in developed vs developing countries in

2018



Source: <http://globocan.iarc.fr>

1.3. Etiology of ovarian cancer

Over the past decades, several risk factors related to the occurrence of OC have been identified. They are classified here into two groups: non-preventable and preventable conditions.

1.3.1. Non preventable conditions

1. Age

OC is an age-related disease, and is considered mainly a postmenopausal disease (13). Increased incidence of this cancer is more pronounced in women over 65 years of age. According to studies, median age at diagnosis is 50–79 years (14).

2. Menstrual related factors

Ovarian tumors are associated with menstrual periods and ovulation cycles (14). Available data support the theory of “incessant ovulation”. Based on this theory, ovulation without interruption can contribute to the incidence of OC by damaging the epithelium of ovaries; therefore, any factor (ex: pregnancy, menstrual disorders, use of oral contraceptives, breastfeeding) that contributes to the reduction of ovulation can have a protective effect against OC (14).

3. Age at menarche and menopause

According to the incessant ovulation hypothesis, early age at menarche and late age at menopause increases risk by increasing the number of ovulatory cycles (14). Conversely, according to the gonadotropin hypothesis (hypothesis suggesting that OC develops from excess stimulation of ovarian tissue by pituitary gonadotropins (FSH, LH et GnRH)), a late age at menopause delays the surge of post-menopausal gonadotropin hormones, possibly reducing risk (13).

4. Parity and infertility

Results of several studies suggest that pregnancy has a protective role against OC (13, 14). Pregnancy causes anovulation and suppresses secretion of pituitary gonadotropins. It is thus consistent with both the ‘incessant ovulation’ and the ‘gonadotropin’ hypotheses. Indeed,

parous women have a 30%-60% lower risk than nulliparous women and each additional full-term pregnancy lowers risk by approximately 15% (13). Regarding the age at first birth, case-control studies have reported an elevated risk associated with late age at first birth (>30 years of age) but not in cohort studies. Recent data also suggests that OC risk does not vary by the time interval between the first and last birth (13).

According to a review of epidemiological studies on OC, infertility (a term that is used to describe a group of biologically distinct conditions ranging from genital tract infections and tubal disturbances to medical conditions such as endometriosis and polycystic ovarian syndrome) appears to be a risk factor in most studies, but not all (13).

5. Familial history and genetic mutations

The strongest risk factor for OC is a family history of breast or OC (14). The risk of developing invasive epithelial OC is increased by approximately 50% among women who have a first-degree relative with a history of OC and by 10% among those who have a first-degree relative with breast cancer (13, 14). It is estimated that approximately 18% of EOC, particularly high-grade serous carcinomas, are caused by inherited mutations that confer elevated risk, the majority in the BRCA1 or BRCA2 gene. Mutations in BRCA1 and BRCA2 genes account for almost 40% of OC cases in women with a family history of the disease (14).

6. Lynch syndrome

Lynch syndrome occurs due to a hereditary mutation in one of the four mismatch repair genes (MHL1, MSH2, MSH6, and PMS2) (15) and MSH2 and MLH1 are the most common mutations in these individuals (16). It is an autosomal dominant cancer predisposition syndrome that is responsible for 10–15% of the total inherited OC cases (14, 17). Most of OC associated with Lynch syndrome are non-mucinous (endometrioid and clear cells) in stage I or II (17, 18).

1.3.2. Preventable conditions : Lifestyle and environmental factors

According to the World Cancer Research Fund (WCRF)(19) report as summarized in **Figure 5**, obesity and adult attained height are associated with a higher risk of OC.

Preventable factors are summarized as following:

1. Breastfeeding

Lactation suppresses secretion of pituitary gonadotropins and leads to anovulation. Both the incessant ovulation and gonadotropin hypotheses would predict lactation reduces the risk of OC especially for long-term duration (13).

2. Hormonal factors

The use of oral contraceptive methods is associated with a lower risk of all histological types of OC whereas hormonal replacement therapy is associated with a higher risk (13).

3. Obesity

Overall, obesity is associated with a higher risk of OC (13, 14, 19)

In postmenopausal women, the predominant source of circulating estrogens is aromatization of androgens in adipose tissue (20, 21). The compelling role of obesity in the pathogenesis of hormone-related cancers, such as endometrial and post-menopausal breast cancers (22), has prompted research on the potential association with OC. However, results on the association between obesity (Body Mass Index, BMI) and OC according to menopausal status are heterogeneous (13).

4. Exercise and physical activity

The general health benefits of exercise are well established and a lower risk of OC might be expected. However, results from epidemiological studies are not consistent (13, 14).

5. Alcohol consumption and cigarette smoking

Studies of alcohol use and OC are inconsistent, with null associations, evidence for higher risk and lower risk of OC with increasing alcohol consumption. Still, analyses by histological subtypes reported a moderately higher risk between regular consumption of alcohol and serous OC risk (13, 14).

Epidemiological studies on smoking and OC concluded that smoking was not a risk factor for OC. However, analyses by histological subtypes reported that smoking appears to be associated with higher risk for mucinous OC in a dose-response manner, but not other subtypes (13, 14).

6. Diet and nutrition

Results from epidemiological studies on diet and OC risk are inconclusive, as indicated in **Figure 5**. The notable exception is intake of vegetables, for which the evidence that higher intakes are associated with lower risk is emerging (23) and to a certain extent also for consumption of whole grain foods and low-fat milk. Regarding vitamin D, experimental studies have shown that vitamin D inhibits cell proliferation in OC cell lines and induces apoptosis (24). However, results from epidemiological studies are inconsistent (13). Similarly, the association between coffee and tea intake is inconclusive (13). In addition, associations between specific fats and oils, fish and meats and certain milk products and OC are inconsistent (13, 14, 19). A summary of investigations on the associations between fatty acids (FA) and OC risk will follow on **Chapter 3**.

Figure 5. World Cancer Research Fund summary on ovarian cancer

2014	DIET, NUTRITION, PHYSICAL ACTIVITY AND OVARIAN CANCER		
		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing		Adult attained height ¹
	Probable		Body fatness ²
LIMITED EVIDENCE	Limited – suggestive	Lactation	
	Limited – no conclusion	Vegetables; fruits; pulses (legumes); red meat; processed meat; poultry; fish; eggs; milk and dairy products; vegetarian and individual level dietary pattern; coffee; tea; dietary fibre; carbohydrates; protein; total fat; saturated fatty acids; monounsaturated fatty acids; polyunsaturated fatty acids; vegetable fat; animal fat; trans fatty acids; dietary cholesterol; alcohol; folate; vitamin A; lycopene; vitamin C; vitamin E; serum vitamin D; lactose; calcium; acrylamide; physical activity; abdominal fatness; energy intake	
STRONG EVIDENCE	Substantial effect on risk unlikely		

¹ Adult attained height is unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linea growth.
² Body fatness marked by body mass index (BMI). The effect may vary in different subgroups such as by tumour type, hormone replacement therapy use, and menopausal status.

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Overall, OC etiology has been widely explored. Although non-preventable factors are well identified, they explain only a small proportion of new OC cases worldwide. Besides, epidemiological investigations have led to the identification of a limited number of preventable lifestyle factors for which there is evidence for possible association with OC occurrence. However, evaluations of the role of nutrition and diet, fat and FA, are still limited and inconclusive with small potential so far for implementation of primary prevention policies. Therefore, one adequate scenario investigating these relationships might be the one providing a large-scale prospective examination along with follow-up for participants, offering both tools

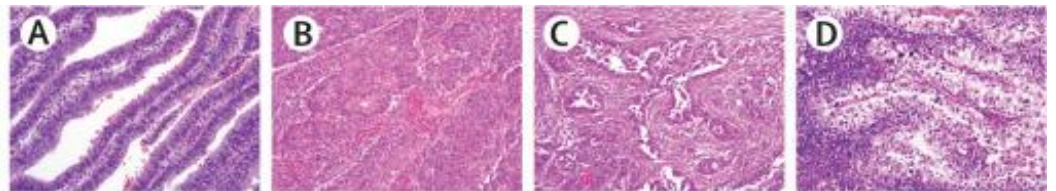
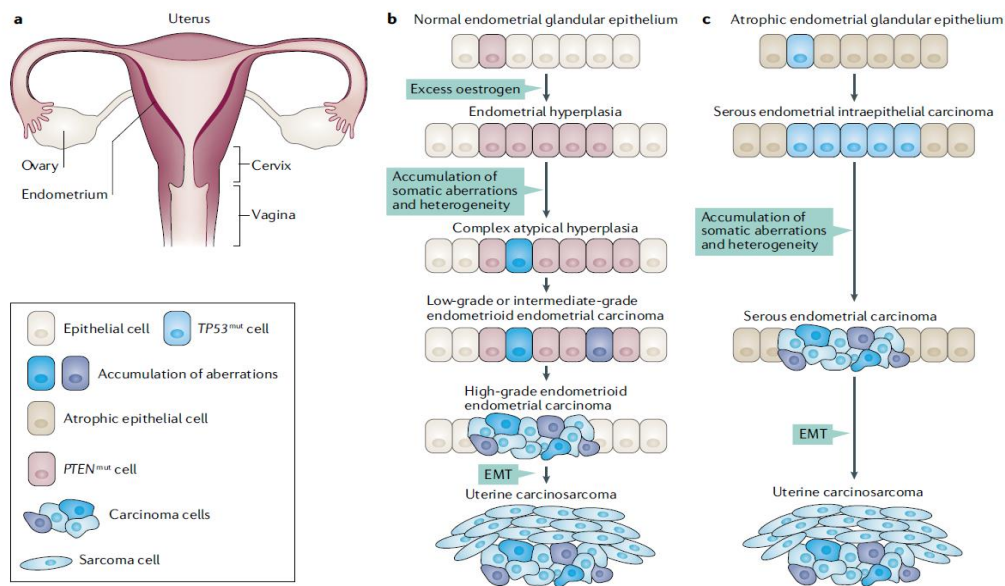
of dietary questionnaires and biomarkers as a complete data in addition to having a sufficient statistical power to detect an association.

Chapter2. Epidemiology of endometrial cancer

2.1. Endometrial cancer development

The endometrium undergoes structural modification and changes in specialized cells in response to fluctuations of estrogen and progesterone during the menstrual cycle. Long-lasting unopposed estrogen exposure leads to endometrial hyperplasia, which increases the chance of development of atypical hyperplasia and eventually cancer (25). The most frequent uterine cancers are endometrial cancer (EC), originating from the uterine epithelium (**Figure 6**). The majority of EC are sporadic whereas 5% of cases are considered to be hereditary and caused by DNA mismatch repair gene mutation (26). ECs are classified into several histological subtypes, including endometrioid EC, serous EC, clear cell EC, mixed EC and uterine carcinosarcoma, which differ in their frequency, clinical presentation, prognosis and associated epidemiological risk factors (27) (**Figure 6**). EC are also divided into two major subtypes: type I EC are usually endometrioid adenocarcinomas, and are linked to excess estrogen in the body. They are associated with mutations in KRAS2 oncogene, PTEN tumor suppressor gene, defects in DNA mismatch repair, and near-diploid karyotype (25). Type I EC occur generally in perimenopausal women, are slow growing, less likely to spread and have favorable prognosis approaching 100% 5-year survival rates (28, 29). Type II cancers include high grade serous carcinomas, undifferentiated and clear cell carcinomas. These cancers are not linked to excess estrogen, highly aggressive and more metastatic. They mostly occur in postmenopausal women and have a poorer prognosis compared to type I (28, 29). Type II are associated with mutations in TP53 and ERBB-2 (HER2/neu) expression, and most are non-diploid (25).

Figure 6. Overview of endometrial cancer origin and development (27)(modified).



Histological type	Endometrioid	Endometrioid	Serous	Clear cell
Histological grade	Low	High	High	High
Metastasis	Uncommon	Lymph nodes Distant organs	Lymph nodes Peritoneal Distant organs	Lymph nodes Peritoneal +/-
Prognosis	Favourable	Poor	Poor*	Poor*†

2.2. Epidemiological trends of endometrial cancer

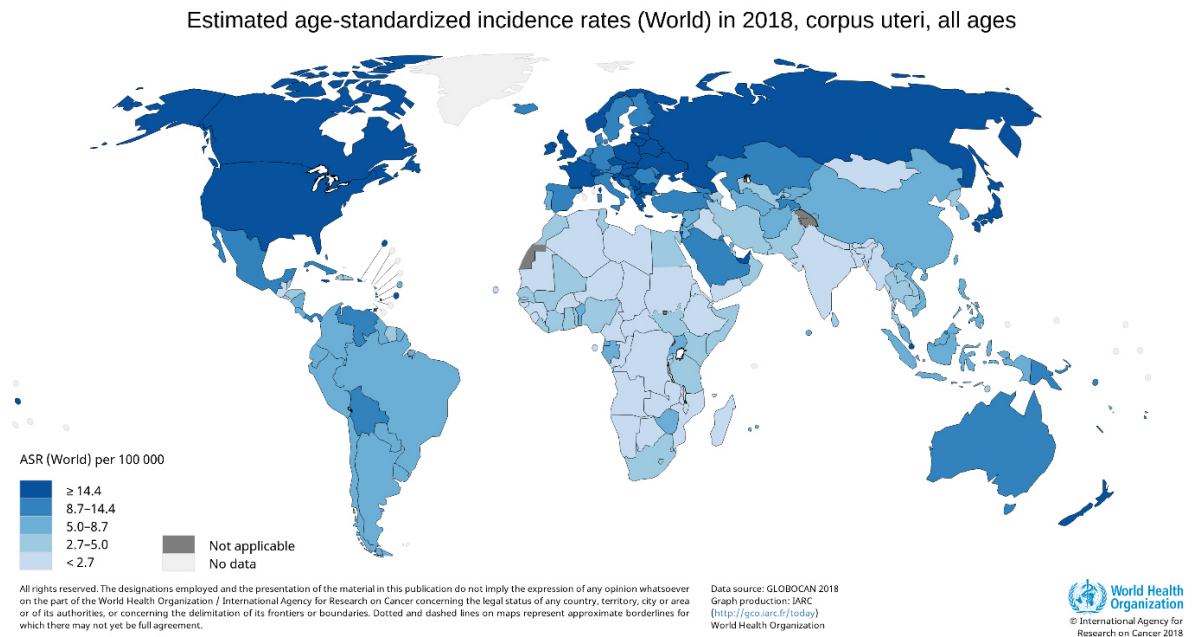
EC with 382,069 new cases and 89,929 deaths in 2018 is the sixth most common cancer in women worldwide and the fourteenth cause of cancer death in women (9). It is the second most common female malignancy in developed world after breast cancer whereas cervix uteri cancer is second most common female malignancy in non-developed world (9). The age-

standardized incidence and mortality rates from EC have been rising steadily in most developed countries over the period 1978–2013, attributed mainly to the obesity and diabetes epidemics, the increasing age of the populations and changes in reproductive behaviors, namely a reduction in parity (30).

EC incidence is also predicted to continue to rise in the coming decades, in particular in low and middle-income countries due to a transition in lifestyle factors (30).

The mean age of women with endometrial carcinoma is 63 years and, above 90% are more than 50 years (31). Most of these patients are diagnosed early, usually at Stage I-II, which carries a favorable outcome with a high 5-year overall survival rate of 96% (32). Abnormal uterine bleeding is the most frequent symptom of EC. All postmenopausal women with vaginal bleeding and those with abnormal uterine bleeding associated with risk factors for EC or hyperplasia (eg, polycystic ovaries, obesity, age over 40 years, erratic cycles, hormone-replacement therapy, tamoxifen use) should undergo further diagnostic endometrial assessment (25).

Figure 7. Age standardized endometrial cancer incidence rates in the world in 2018



Source: <http://globocan.iarc.fr>

2.3. Etiology of endometrial cancer

Over the past decades, several risk factors related to the occurrence of EC have been identified. As for OC, they are classified here into two groups: non-preventable and preventable conditions.

2.3.1. Non-preventable conditions

1. Age

EC is found to be positively correlated with older age (33). As EC is more common in post-menopausal women than in premenopausal women, over 90% of the cases are diagnosed after the age of 50 years (26).

2. Race

Data from Western countries showed differences in the incidence of EC and mortality rate among races. White women have a higher risk of developing EC than women belonging to other ethnic groups (34). However, in comparison with other races, the mortality rate in white women is the lowest. Different incidence rates of EC among races could be due to differences in life-style, socioeconomic status, and genetic predisposition to developing EC (35).

3. Early menarche and late menopause

Because early menarche and the late menopause increase the number of menstrual cycles, and consequently the total exposure time to estrogens, early menarche and late menopause have been associated with a higher risk of EC (36).

4. Family history

About 5% of EC cases have a family history of the disease among first degree relatives (37). Family history of EC is associated with disease higher risk from two to three fold among premenopausal women (38). In women less than 50 years old, about 9% of EC is due to mutations in mismatch repair genes (MSH1, MSH2, MSH6), which lynch syndrome (39).

In carriers of BRCA1 mutations, the overall higher risk of EC and other cancer except breast and ovary is small (40) while no increase was reported BRCA2 mutation carriers (41).

5. Lynch syndrome

Lynch syndrome patients are at higher risk for a number of different malignancies, but most commonly develop colorectal and EC (42).

6. Tamoxifen use

Tamoxifen is a selective estrogen receptor modulator often used to treat women with an estrogen receptor positive breast cancer (43). Tamoxifen stimulates endometrial proliferation and the thickness of the endometrium increases depending on the duration of the drug use (44). EC risk increases with the duration of tamoxifen use compared to non-users. In addition, long term Tamoxifen use is usually associated with a poor prognosis and poor survival rate (45, 46).

7. Parity and infertility

Lower parity and/or nulliparity were reported to be associated with higher risk of EC up to four-fold, while multiparity has been associated with lower risk, up to 70%. Furthermore, any additional birth among parous women (after the birth of the second child) decreased the risk of developing the disease by 10 % for every new child (47). This is because parity causes an alteration in the hormonal balance towards increasing progesterone and decreasing estrogen which suppresses endometrial mitotic activity (47).

Infertility has been associated with higher risk of developing EC at a younger age (39). Indeed, the majority of young patients with EC (<40 years) suffers from chronic anovulation due to elevated serum estrogen levels (48). Thus, women with polycystic ovary syndrome and women with estrogen-secreting ovarian tumors are more prone to have EC especially in their reproductive life (49).

8. Diabetes

Diabetes is positively associated with EC (50). However, this could be due to confounder's effect, as women with type 1 diabetes are more likely to be nulliparous, to have irregular menstruation, fertility disorders and be obese, all risk factors for EC (51).

2.3.2. Preventable conditions: lifestyle and environmental factors

According to the WCRF report as summarized in **Figure 8**, obesity, adult attained height, and glycemic load are associated with a higher risk of EC, physical activity and coffee with a lower risk (52).

Lifestyle factors associated with EC are summarized as following:

1. Obesity

Obesity may increase EC incidence, with obese women having a twofold to fivefold higher risk of developing EC compared with normal weight women. In general, obesity is associated with higher levels of circulating estrogens in postmenopausal women, likely accounting for the higher risk of EC (53).

2. Smoking and alcohol

Smoking is considered to have a protective effect on EC. This might be attributed to its anti-estrogenic effect (54).

High consumption of alcohol-containing beers, wines, and white spirits was not associated with EC risk (55).

3. Hormonal factors

Use of contraceptive pills containing estrogen and progesterone has been associated with lower EC risk (25). After menopause, for women taking HRT (estrogen and progesterone), progesterone counteracts the adverse effects of estrogen and led to a lower EC risk (25).

4. Exercise and physical activity

Sedentary behavior is positively associated with more than ten types of cancer; including EC, whereas, exercise and physical activity are associated with a lower risk (56).

5. Diet and nutrition

The evidence of an association between EC risk and specific dietary components is limited, as indicated in **Figure 7** (52). Limiting energy-dense foods by limiting the carbohydrates macronutrients is also an important aspect of improving the underlying metabolic abnormalities (such as obesity and insulin resistance) that promote endometrial pathology (57, 58).

Vegetarian diet, fruits and nutrients such as fibers and vitamins are associated with a reduced risk of EC (59-61). Regarding fat and FA, there is some evidence of a positive link between high dietary fat intake and EC. As a matter of fact, higher fat intake was linked to increased plasma estradiol, insulin secretion and Insulin Growth Factor (IGF) levels, and inflammation markers (including C-reactive protein, intercellular adhesion molecule-1, and interleukin-6). Hence, dietary fat intake may promote EC development through unbalanced hormone, insulin and IGFs, and inflammation systems (62-64). A summary of investigations on the associations between FA and EC risk will follow on **chapter 3**.

Figure 8. World Cancer Research Fund summary on ovarian cancer (52)

2013	DIET, NUTRITION, PHYSICAL ACTIVITY AND ENDOMETRIAL CANCER		
		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing		Body fatness ¹
	Probable	Physical activity ² Coffee ³	Glycaemic load Adult attained height ⁴
LIMITED EVIDENCE	Limited – suggestive		Sedentary habits ⁵
	Limited – no conclusion	Cereals (grains) and their products; fruits; vegetables; pulses (legumes); soya and soya products; red meat; processed meat; poultry; fsh; eggs; milk and dairy products; dietary fibre; total fat; animal fat; saturated fatty acids; cholesterol; tea; glycaemic index; protein; retinol; beta-carotene; folate; vitamin C; vitamin E; multivitamins; alcohol; acrylamide; dietary pattern; and lactation	
STRONG EVIDENCE	Substantial effect on risk unlikely		

- 1 The Panel interpreted BMI (including BMI at age 18-25 years), measures of abdominal girth, and adult weight gain as interrelated aspects of body fatness as well as fat distribution.
- 2 Physical activity of all types: occupational, household, transport and recreational.
- 3 The effect is found in both caffeinated and decaffeinated coffee and cannot be attributed to caffeine.
- 4 Adult attained height is unlikely to modify the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth.
- 5 Sedentary habits as marked by sitting time.

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Understanding EC etiology would be important for public health strategies aiming to prevent this cancer. Among risk factors that have been identified so far, a large number are non-preventable or explain only a small proportion of EC risk. As for other risk factors that have not been sufficiently studied yet, nutrition and diet, particularly fats and FA, deserve further investigation particularly that they are potentially preventable and affect a variety of diseases. Such an investigation would strongly benefit from a study design involving a large-scale prospective examination, with sufficient statistical power, along with follow-up for

participants and dietary assessment tools that involve both questionnaire data and biomarker measurements.

Chapter 3. Fatty Acids, Structures and functions

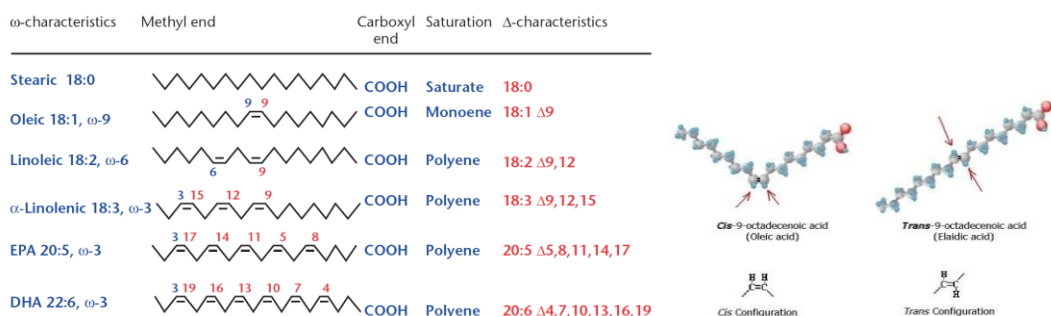
3.1. Structures and functions of fatty acids

Fatty acids- an overview

FA are carboxylic acids with an aliphatic chain, which is either saturated or unsaturated. Chemical structures of examples of FA from different classes and configurations (*cis* vs *trans*) as well as the origin are presented in **Figure 9** and **Figure 10**.

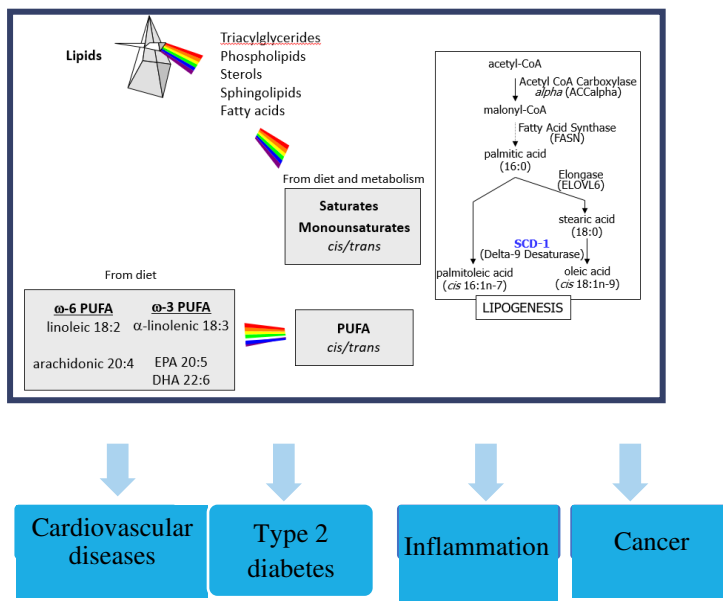
FA have diverse functions in cells that range from structural “building blocks” of cell membranes to suppliers of energy and signaling molecules. Thus, they can influence membrane fluidity (or order) and function, and cell and tissue responses through the regulation of intracellular signaling pathways, transcription factor activity, and gene expression depending on their type and degree of unsaturation (Figure 7 and 8) (65, 66). Therefore, FA might impact health and disease risk like cardiovascular diseases (CVD), metabolic diseases such type II diabetes, inflammatory diseases and cancer. General functions of FA are presented in **Figure 10**.

Figure 9. Fatty Acids: Structures and configurations(67) (modified)



Structure of different unbranched fatty acids with a methyl end and a carboxyl (acidic) end. Stearic acid is a systematic name for a saturated fatty acid with 18 carbon atoms and no double bonds (18:0). Oleic acid has 18 carbon atoms and one double bond in the ω -9 position (18:1 ω -9) where ω could also be replaced by n. Eicosapentaenoic acid (EPA), with multiple double bonds, is represented as 20:5 ω -3. This numerical scheme is the systematic nomenclature most commonly used. The second part of the figure, show the *cis* vs *trans* configurations of the double bonds

Figure 10. Fatty acids: origins and functions (47)



FA are the building blocks of the lipids. Saturated and monounsaturated originate from both diet and metabolism, whereas polyunsaturated and *trans* fatty acids originate exclusively from diet. FA have divergent effects on human health like CVD, type 2 diabetes, inflammation and cancer.

Saturated and monounsaturated Fatty acids

Structures:

Saturated Fatty Acids (SFA) are FA with no double bond. The dietary sources of these FA are mainly palm oil, coconut oil, cocoa and animal-derived fat (butter, meat – such as fatty tallow of beef, pork and lamb, processed meats). Most common SFA deriving from these dietary sources are the even-numbered SFA: palmitic acid (16:0 where 16 is the number of carbon atoms), stearic acid (18:0) and myristic acid (14:0). Odd-chain SFA, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), derive mainly from dairy fats (66).

Cis-Monounsaturated Fatty Acids (*cis*-MUFA) are FA with one double bond in a *cis* configuration. The most prevalent *cis*-MUFA in human diet is oleic acid (18:1n-9 where 18 is the number of carbon atoms, n (or ω) is the number of double bonds, and 9 is the position of the double bond counting from the methyl group end (**Figure 9**)), followed by palmitoleic acid

(16:1n-7). Oleic acid is derived from many plant oils like olive oil, animal derived fats like lard and butter while palmitoleic acid is abundant in seed oils (Macadamia and sea buckthorn oil), fatty fish and fish oils, and is scarce in plant oils and animal fat (66).

Endogenous synthesis:

SFA and *cis*-MUFA do not come only from diet, but can also be synthesized *de novo* in human liver and adipose tissue by Acetyl-CoA carboxylase (ACC), Fatty Acid Synthase (FAS), and Stearoyl-CoA desaturase (SCD). ACC catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA. FAS is a multi-enzyme protein, encoded by the FASN gene, that catalyzes FA synthesis. SCD is an endoplasmic reticulum-bound enzyme that catalyzes the Δ^9 -*cis* desaturation of saturated fatty acyl-CoAs, the preferred substrates being palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively. These MUFA are used as substrates for the synthesis of triglycerides, wax esters, cholesteryl esters and membrane phospholipids.

Functions:

Even-numbered SFA may raise total and LDL cholesterol blood concentrations, increase coagulation, inflammation and insulin resistance whereas odd-chain SFA are associated with lower risk of type II diabetes, Coronary Heart Disease (CHD), CVD (66) and cancer (28-37).

Cis-MUFA are known to have modest effects on inflammatory processes (68) and little effect on lowering blood pressure (69), glucose control, insulin sensitivity, obesity (70) and cancer (32,41-44).

The monounsaturated to saturated FA ratios (where DI_{16} and DI_{18} are the desaturation indexes of palmitoleic to palmitic and oleic to stearic respectively) have been linked to a variety of diseases (**Figure 10**) (66, 71). For example, in experimental studies, SCD1 is known to be a driver of abnormalities that lead to the development of metabolic disorders, such as diabetes, hyperlipidemia, hepatic steatosis, and obesity-related heart diseases in mice models (72, 73).

SCD1 has been also associated with over-all cancer development, progression, cell survival and metastatic potential (74). In epidemiological studies, a higher DI measured in pre-diagnostic blood samples was reported to be associated with a higher risk of breast cancer (75-77), suggesting that, in accordance with experimental data, increased hepatic stearoyl-CoA desaturase expression/activity may be related to higher risk of breast cancer. In addition, in a clinical trial in the US, decreasing levels of DI₁₆ and DI₁₈ were associated with a progressive reduction in breast density but only in obese women (78).

These findings suggest that SFA, MUFA and/or the endogenous synthesis of MUFA might have protective or harmful effects for health depending on their class or type.

Polyunsaturated fatty acids

Structures:

N-6 Polyunsaturated Fatty Acids (n-6 PUFA) are FA with two or more double bonds in a *cis* configuration and having the first double bond on position 6 counting from the methyl end. The most abundant n-6 PUFA is linoleic acid (18:2n-6), followed by arachidonic acid (20:4n-6). Linoleic acid is an essential FA, derived only from diet and mainly from seeds, nuts vegetable oils (safflower oil, maize oil, sunflower oil and soybean oil), meat and eggs (66, 79). Arachidonic acid is found in animal food such as meat and eggs (66). Linoleic acid is the metabolic precursor of arachidonic acid (**Figure 11**) (65, 66).

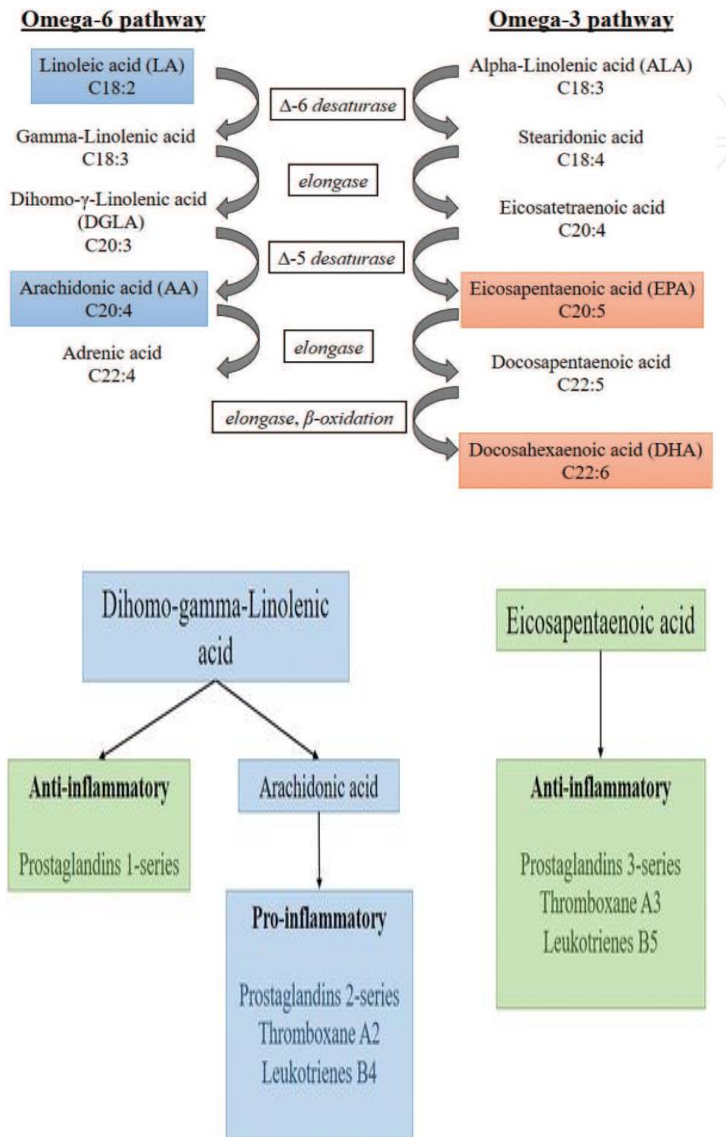
N-3 PUFA are FA with two or more double bonds in a *cis* configuration and having the first double bond on position 3 counting from the methyl end. The essential α -linolenic acid (18:3n-3) derive mainly from seeds (flaxseeds and flaxseed oils) and nuts. α -Linolenic acid is the precursor of long-chain PUFA (LC-PUFA), eicosapentanoic acid (EPA, 20:5n-3) and then

docosahexanoic acid (DHA, 22:6n-3) (65, 66). EPA and DHA are found in seafood particularly fatty fish and fish oil supplements. α -Linolenic acid uses the same metabolic pathway and the same enzymes as linoleic acid, leading to a direct competition between n-6 and n-3 PUFA (**Figure 11**) (65, 66).

Functions:

N-6 arachidonic acid is the precursor for the synthesis of pro-inflammatory eicosanoids, while n-3 EPA is a precursor for the synthesis of anti-inflammatory eicosanoids (65) (**Figure 11**). Divergent associations were reported in epidemiological studies between n-6, n-3 PUFA and cancer including pancreatic, prostate, breast and colorectal cancers(74, 80-85): The ratio n-6/n-3 PUFA which considers the balance of intake between these two families is higher in western diet compared to other diets including Asian diet (86) and is associated with higher risks of several cancers (78, 87). These findings suggest that PUFA might have protective or harmful association with NCDs depending on their class or type; however, further studies are needed to confirm these associations.

Figure 11. N-6 and N-3 Polyunsaturated fatty acids pathways and roles(65) (modified).



Trans fatty acids

Structures:

Trans fatty acids (TFA), unsaturated FA in a *trans* configuration, are divided into two groups: the ruminant TFA (rTFA) deriving from the rumen metabolism, and the industrial TFA (iTFA) arising through cooking and particularly hydrogenation processes which increase the shelf life and flavor stability of foods. The most frequent rTFA found in milk and meat are *trans*-vaccenic acid (*trans* 18:1n-11) and the Conjugated linoleic acid (CLA) (*cis*-9, *trans*-11 and *trans*-10,*cis*-12 CLA isomers). The most frequent iTFA is elaidic acid (*trans* 18:1n-9) derived from industrial processing and deep-frying foods (66).

Functions:

Higher consumption of ultra-processed foods was associated with higher risks of cardiovascular, coronary heart, and cerebrovascular diseases (88). In addition, 10% increase in the proportion of ultra-processed foods in the diet was associated with a significant increase of greater than 10% in risks of overall cancers (89). These findings suggest that iTFA are harmful for health.

Over-all, depending on their types and classes, FA might impact health, well-being and NCDs risk like CVDs, metabolic diseases such type II diabetes, inflammatory diseases and cancer.

3.2. Fatty Acids and ovarian and endometrial cancers : epidemiological studies

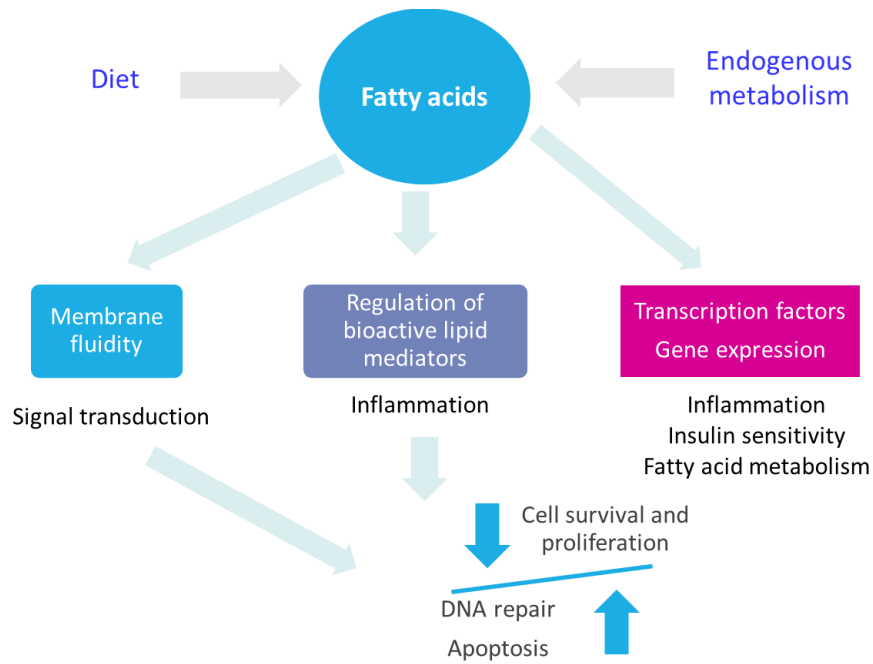
As previously reported, FA impact NCDs risk like CVD, metabolic diseases such type II diabetes, inflammatory diseases and cancer.

Regarding cancer, there is a strong biological plausibility underlying the association of FA to cancer development (66, 90). Alterations in cellular metabolism and energetics are hallmarks of cancer. One of the earliest observations of altered tumor metabolism was increased aerobic glycolytic flux, termed the Warburg effect (91-93). Warburg effect with aerobic glycolysis efficiently produces ATP synthesis and consequently promotes cell proliferation by reprogramming metabolism to increase glucose uptake and stimulating lactate production(94). High-proliferating cancer cells use increased FA synthesis to support the rate of cell division(95).

It is well documented that cancer cells show specific alterations in different aspects of lipid metabolism. These alterations can affect the availability of structural lipids for the synthesis of membranes, the synthesis and degradation of lipids that contribute to energy homeostasis and the abundance of lipids with signaling functions. Besides an alteration in FA in cancer cells, dietary FA, according to their types, can affect numerous cellular processes, including cell growth, proliferation, differentiation, and motility. Their biological activities may be grouped as regulation of membrane structure and function; regulation of intracellular signaling pathways, transcription factor activity, and gene expression; and regulation of the production of bioactive lipid mediators. Through these effects, FA influence cancer risk (66, 90). Dietary fat appears to act primarily during the promotional stage of carcinogenesis in most cancer models (96). The biological plausibility between FA and cancer is presented in **Figure 12**.

As my PhD focused on FA and OC and EC, the following paragraphs will report in details the epidemiological association of FA with the risk of these cancers.

Figure 12. Fatty acids and cancer development: Biological plausibility (66)



3.2.1. Fatty acids and ovarian cancer

In experimental studies, dietary fat has been hypothesized to affect ovarian carcinogenesis primarily through hormone-related mechanisms. In fact, high fat intake may expose the ovarian epithelium to high levels of endogenous estrogens, which may trigger the development of EOC through cell damage and proliferation (20, 97, 98)

In epidemiological studies, data remain conflicting. A review focused on the dietary determinants of EOC reported inconsistent and inconclusive results between fats, oils and EOC risk, but a typical Western diet, which is high in meats, fats, sugar and salt, and low in vegetables, may be positively associated with the development of EOC (23). A systematic meta-analysis by the World Cancer Research Funds concluded that there was “limited” evidence for a link between total fat, saturated/animal fat and OC (19). A meta-analysis including case-control and cohort studies suggested a lack of evidence for associations between dietary fat and FA intakes and EOC risk(99) while another meta-analysis indicated that high consumption of total, saturated and *trans*-fats are associated with higher risk of OC, with different histological subtypes having different susceptibility to dietary fat (100). Results from a Chinese case-control study suggested that the risk of OC declined with increasing consumption of vegetables and fruits and increased with high intakes of animal fat and salted vegetables(101). Findings from an American case-control study suggested that higher intake of n-3 PUFA may be protective for EOC overall and endometrioid tumors in particular, whereas greater consumption of *trans* fat may increase risk of EOC overall (102). In two cohort studies in the US, the Nurses' Health Study (NHS) and NHSII, no clear associations between dietary fat and OC risk were reported (103). Data from the EPIC and the Netherlands Cohort Studies reported a greater risk of OC associated with a higher intake of saturated fat (104). In the EPIC study only, higher intake of PUFA was associated with higher risk of OC (105).

Higher fat intake from animal sources, but not from plant sources, was associated with a greater risk of OC in the National Institutes of Health-American Association of Retired Persons (NIH-AARP) diet and health study (106).

As listed above, data from epidemiological studies on dietary FA and OC risk are discrepant. In addition, epidemiological data on biomarkers of exposure to FA and OC risk are scarce. Thus, additional prospective studies, with high statistical power, that integrate both dietary and biomarkers exposure to FA are needed.

3.2.2. Fatty acids and endometrial cancer

In vivo and *in vitro* studies suggested that several components of diet, especially lipids including saturated fatty acid, unsaturated fatty acid, and cholesterol intake might influence the proliferation of EC cells by modulating the production, metabolism, and excretion of endogenous hormones (57, 58, 107-109) .

A systematic meta-analysis by the World Cancer Research Funds concluded that there was “limited” evidence for a link between total fat, saturated/animal fat and EC (65). Data from the EPIC and the Nurses' Health Study (NHS) and NHSII reported a higher risk of EC associated with a higher intake of total fat and monounsaturated fat (110). Data from a meta-analysis suggested a lack of association between total dietary fat intake and EC risk (111). Results from another meta-analysis of 7 cohorts and 14 case-control studies suggested that higher MUFA intake was associated with lower EC risk; total fat and saturated fat intake were associated with a higher risk of EC in the case-control studies of this meta-analysis while no significant associations were suggested with PUFA and linoleic acid (112). Another meta-analysis of 8 case-control and 4 cohort studies suggested that intake of n-3 PUFA may be inversely associated with EC risk at some level of evidence, although the exact relationship,

especially for fish intake, needs further characterization(113). A review on the effects of the dietary and nutrient intake on gynecologic cancers counting EC concluded that long-term consumption of pro-inflammatory foods, including saturated fat, carbohydrates and animal proteins is a risk factor for these cancers (114). A case-control study in the US reported a reduced EC risk associated with a diet high in plant foods (115). Another case-control study in the US suggest that dietary intake of the long-chain PUFA; EPA and DHA in foods and supplements may have protective associations against the development of EC (116) while a case-control study in Mexico reported no association between FA and EC risk (109, 117) . In a nutrient wide association, a negative association was reported between total fat and MUFA and EC risk in EPIC while no association was reported between dietary fat and EC risk in the NHS and NHSII cohorts (110). In the Women Health Initiative (WHI) study in the US, LC n-3 PUFA intake was associated with reduced EC risk in normal-weight women (118).

Data from epidemiological studies on dietary FA and EC risk are discrepant and heterogeneous between prospective and retrospective studies, large and small number of cancer cases. In addition, there is no epidemiological data on biomarkers of exposure to FA and OC risk. Thus, additional prospective studies, with high statistical power, that integrate both dietary and biomarkers exposure to FA are warranted.

***Chapter 4. Dietary and circulating fatty acids and
ovarian cancer risk in EPIC***

4.1. Background & aims

In Europe, OC is the fifth most common cancer and the sixth cause of death from cancer in women. Few modifiable risk factors are known. As its incidence is rising worldwide, prevention strategies are urgently needed.

FA have been postulated to affect important risk factors for OC such as obesity, estrogens levels, insulin resistance and inflammation and to have direct tumorigenic effects in animal and *in vitro* models; thus, they may be involved in OC development. However, epidemiological studies are scarce and findings heterogeneous, probably due to the complexity of FA, to measurement errors in the FFQ and to the study designs.

The aims of this study were to:

- 1- Determine the associations between dietary (estimated through FFQ) and plasma phospholipid FA (PL-FA, biomarkers measured through gas chromatography) and OC risk, overall and by different levels of stratifications.
- 2- Determine the dietary sources of FA and their link with OC risk.

4.2. Materials & Methods

4.2.1. Population study, the EPIC cohort

The EPIC cohort is an on-going multicenter prospective cohort study, mainly designed to study the relationship between nutrition and cancer (119). Over 521,000 participants, aged between 25 and 70 years, were recruited between 1992 and 2000 across 23 centers spanning

10 European countries including: France, Germany, Greece, Italy, The Netherlands, Spain, the United Kingdom, Sweden, Denmark and Norway (120).

Briefly, dietary information, as well as socio-demographic, and lifestyle data were collected at enrolment from all study participants by administration of country-specific questionnaires. Standardized socio-economic and lifestyle questionnaires were completed for education, smoking, physical activity, alcohol intake, oral contraceptive and hormonal therapy.

Nutrient databases and the 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. The ENDB database (121) was used as a basis to match the EPIC data with FA isomers, using the National Nutrient Database for Standard Reference of the United States (NNDSS; further referred to as USDA table) (121, 122). Amounts of FA were obtained through this extra USDA matching. Groupings of FA are as presented in **Table 1**, as below.

Table 1. Groupings of fatty acids

Grouping of fatty acids	List of individual fatty acids of each grouping
SFA	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
<i>Cis</i> -MUFA	16:1n-7, 16:1n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1
n-6 PUFA	18:2, 18:3, 20:2, 20:3, 20:4
n-3 PUFA	18:3, 20:3, 20:5, 22:5, 22:6
LC n-6 PUFA	20:2, 20:3, 20:4
LC n-3 PUFA	20:3, 20:5, 22:5, 22:6
Rtfa	18:1n-7, CLA
Itfa	16:1n-9, 18:1n-9, 18:2n-6, 18:3n-3

Baseline anthropometric measurements and peripheral blood samples were collected at baseline in 80% of the recruited cohort participants prior to cancer onset, providing invaluable biomarker measurements, as detailed in **Table 2**. In most centres, body weight and standing height were measured and applied to calculate the body mass index (BMI, weight/height squared, kg.m⁻²). Procedures for sample collection, processing and storage are described in detail elsewhere (120). Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer and from all local institutions.

Table 2. Number of EPIC subjects by country with questionnaires information and availability of blood samples.

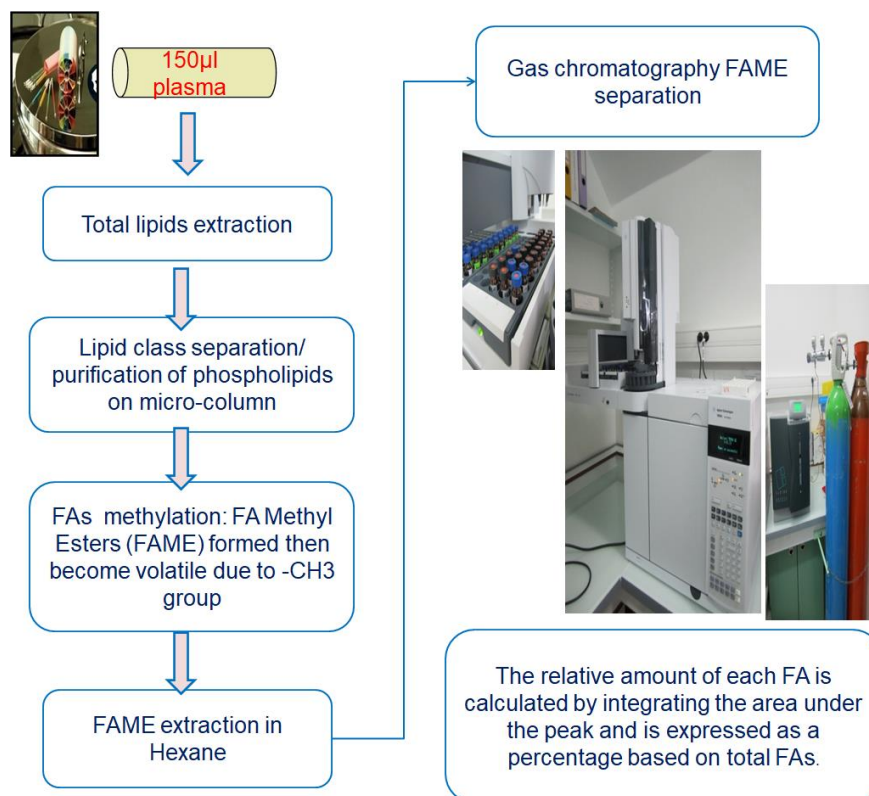
	Study subjects	
Country	Questionnaire	Questionnaire +Blood
France	74,524	28,083
Italy	47,749	47,725
Spain	41,440	39,579
U.K.	87,942	43,141
The Netherlands	40,072	36,318
Greece	28,555	28,483
Germany	53,091	50,678
Sweden	53,826	53,781
Denmark	57,054	56,131
Norway	37,215	31,000
Total	521,468	414,889

2

Measurement of plasma phospholipid fatty acids biomarkers:

Gas chromatography with an FID (Flame Ionization Detector) detector is the methodology used to determine plasma phospholipid (PL) concentrations of sixty FA. All laboratory analyses were performed by IARC laboratory technicians. The methodology is summarized in the graph below (**Figure 13**) and detailed elsewhere (76).

Figure 13. Quantification of circulating fatty acids



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.

4.2.2. Study designs & statistical analyses

This study was conducted in two designs:

- An **EPIC-wide design** was conducted to assess the association between estimated dietary intakes of FA and the risk of OC. Fatty acid intakes for 1486 cases and 321,867 non-cases were quantified through FFQ. Multivariable Cox proportional hazards regression models were used to estimate relative risk of OC across quintiles of FA. Due to the number of tests performed, q-values were calculated using the False Discovery Rate (FDR) of the Benjamini-Hochberg procedure. We also estimated the associations between main dietary sources of FA (as continuous variables) and OC risk.

- A **case-control design nested within the EPIC** study was conducted to assess the association between plasma phospholipid FA and the risk of OC. A total of 1,075 incident cases of first incident invasive EOC were identified among cases who had completed the dietary questionnaire and provided a baseline blood sample. Samples from Denmark

were not included in this analysis, leading to 633 cases. For each case, two controls were matched to cases on study center, age at blood donation, time of the day of blood collection, fasting status, menopausal status, and menstrual cycle phase for premenopausal women, current use of oral contraceptives or HRT. The FA composition in the phospholipid fraction was measured by gas chromatography in plasma samples collected at recruitment. Multivariable conditional logistic regression models were used to estimate relative risk of OC across tertiles of plasma FA.

For both designs, models were adjusted for the duration of oral contraceptive use, parity, menopausal status at enrolment and total energy intake. Models were stratified by menopausal status (pre vs post), histological subtypes of EOC (serous, mucinous, endometrioid and clear cell), grades (I, II, III) and BMI (underweight, normal, overweight and obese).

4.3. Results

A positive association was found between EOC risk and intakes of iTFA (HR comparing 5th with 1st quintile $_{Q5-Q1}$ =1.34, 95% CI=1.06-1.67, p_{trend} =0.01, q-value=0.04) mainly driven by elaidic acid (HR $_{Q5-Q1}$ = 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 linoleic acid (HR $_{Q5-Q1}$ =1.34, 95% CI=1.07-1.67, p_{trend} <0.001, q-value=0.005) mainly driven by the contribution of deep-frying fat (HR $_{Q5-Q1}$ =1.10, 95%CI=1.01-1.21), and a positive association with α -linolenic acid (HR $_{Q5-Q1}$ =1.29, 95% CI=1.05-1.58, p_{trend} =0.007, q-value=0.002) 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).

In our subset of analysis, a borderline positive trend was reported between EOC risk and plasma phospholipid elaidic acid (OR comparing 3rd with 1st tertile $_{T3-T1}$ =1.39, 95% CI=0.99-

1.94, $p_{\text{trend}}=0.06$) 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 ($\text{OR}_{\text{T3-T1}}=1.30$, 95% CI= 0.98-1.72, $p_{\text{trend}}= 0.06$).

4.4. Conclusion

Most all fatty acid intakes in this study are in the recommended range, e.g. total SFA (10.84% of total energy in this study versus <10% recommended), MUFA (15.8% vs 15-20% recommended), n-6 PUFA (5.37% vs 5-8% recommended), and iTFA (0.87% vs 1% recommended). In contrast, n-3 PUFA were lower than those recommended (0.31% vs 1-2% recommended) (123, 124). Levels of EPA (60mg/d) and DHA (100mg/d) in this study were lowest than those recommended in the world: > 500 mg/d EPA+DHA and in Europe: 250mg/d EPA+DHA (125).

Even with a mean of intake <1% responding to the WHO actual recommendations, our results suggest that higher dietary intakes and circulating levels of iTFA might be associated with greater risk of EOC. TFA may have decreased in processed foods, but may still be high in certain vulnerable groups in the population (126). Voluntary reduction of TFA in foods has been reported to be an ineffective strategy in several European countries (127), suggesting that a legislative iTFA ban may be the only effective strategy to minimize the exposure to iTFA.

In addition, higher intakes of linoleic acid and α -linolenic acid originating mainly from deep frying fat, may be associated with greater risk of EOC. 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 compounds occurring in foods exposed to deep

frying fat and margarine (such as aldehydes, oxidized lipids, heterocyclic compounds, TFA, polymers, sterol derivatives, acrylamide, and acrolein (128)).

Our findings propose to eliminate iTFA from industrialized and deep-fried food. This is in line with WHO REPLACE initiative (129) aiming to globally ban iTFA intake by 2023 in order to reduce chronic diseases including EOC.

4.5. Scientific article

“Dietary and circulating fatty acids and OC risk in the European Prospective Investigation into Cancer and Nutrition”, by Yammine S. G. *et al.*, has been accepted for publication by Cancer Epidemiology Biomarkers and Prevention, on 6 April 2020 I am the first author of this publication.

My roles in the development of this study and the production of the manuscript were to: participate to the analytical strategy, conduct all statistical analyses, write the manuscript and revise it according to the reviewers’ comments.

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Original article, in press, CEBP,2020

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

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Disclosure

The authors have declared no conflicts of interest.

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

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 (9). As the incidence of ovarian cancer is rising worldwide, prevention strategies are urgently needed; however, few preventable factors have been identified (130). 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) (23).

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 (19). 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 (104). In the EPIC study only, higher intake of polyunsaturated fatty acids was associated with higher risk of EOC(105). 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 (106).

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 (131). The study design, recruitment procedures and data collection have been described previously (119). 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 (120).

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-standardised procedure was used, adopting nutrient values from ten national food composition databases of the respective EPIC countries (121, 132). ENDB was used to match the EPIC data with fatty acid isomers using the National Nutrient Database for Standard Reference of the United

States (NNDSSR; further referred to as USDA table) (133). 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(134). 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 ($p < 0.01$), respectively¹⁵. 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 acids¹⁶. 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 (135). Controls were matched to cases on study center, age at blood donation (± 1 year), 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 (76). 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 ($\mu\text{mol/l}$).

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 (136).

Statistical analyses

In the descriptive statistics for socio-demographic and lifestyle characteristics and dietary intake of fatty acids, frequencies were reported for the categorical variables and means \pm standard deviations (SD) were calculated for the continuous variables. Hazard ratios (HR) 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 enrolment. The retained multivariable model was adjusted for duration of oral contraceptive use (never use; use <5 years; use \geq 5 years; missing), parity (number of live 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 (history/duration of breastfeeding, ever use of postmenopausal hormones, history of unilateral ovariectomy, BMI, physical activity, tobacco smoking, education status, and intake of 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. Multiple testing correction was performed using

Q-values were calculated using the false discovery rate of the Benjamini-Hochberg procedure²⁰.

Additionally, 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 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 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 risks 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 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-Q1}=1.34, 95% CI=1.06-1.67, $p_{\text{trend}}=0.01$, $q\text{-value}=0.04$) mainly driven by elaidic acid (HR_{Q5-Q1}= 1.29; 95% CI=1.03-1.62; $p_{\text{trend}}=0.02$, $q\text{-value}=0.06$). A positive association was also reported between EOC risk and intakes of total PUFA (HR_{Q5-Q1}=1.41, 95% CI=1.13-1.77, $p_{\text{trend}}<0.001$, $q\text{-value}=0.005$), mainly driven by linoleic acid (HR_{Q5-Q1}=1.34, 95% CI=1.07-1.67, $p_{\text{trend}}<0.001$, $q\text{-value}=0.005$), and α -linolenic acid (HR_{Q5-Q1}=1.29, 95% CI=1.05-1.58, $p_{\text{trend}}=0.007$, $q\text{-value}=0.002$) (**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-T1}=1.39, 95% CI=0.99-1.94, $p_{\text{trend}}=0.06$) 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-T1}=1.30, 95% CI=0.98-1.72, $p_{\text{trend}}=0.06$) (**Table 3**).

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

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 ($p_{\text{heterogeneity}} = 0.04$). All p for 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 histological subtypes of EOC.

iTFA consumption is associated with increased all-cause mortality²⁴ and the WHO encourages the elimination of these fatty acids from the diet²⁵. TFA may have decreased in processed foods, but their intake may still be high in certain countries or vulnerable groups in the population²⁶. 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 EOC²⁷. 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 EPIC⁶ and the NIH-AARP Diet and Health studies⁷, 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 NHS²⁸. 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 polyunsaturated fatty acids compared with the limited conversion of alpha-linolenic acid to its longer chain derivatives²⁹.

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 -linolenic 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			Nested case-control study			p**
	Epithelial ovarian cancer cases	Non cases*		Epithelial ovarian cancer cases	Controls	
N= 323,376	n=1,486	n=321,890		n=633	n=1,248	
Anatomical subtypes, number, (%***)						
Serous	79 (53.4)	-		341 (53.7)	-	-
Mucinous	91 (6.1)	-		37 (5.8)	-	-
Endometrioid	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)***						
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

Phospholipid fatty acids###
(% of total fatty acids)
Mean±SD***

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
<i>n</i> -6 PUFA (g/day)	11.5 (5.6-21.3)	11.4 (5.8-22.4)	<i>n</i> -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
<i>n</i> -6 long-chain PUFA (mg/day)	23.5 (6.5-66.2)	24.2 (5.7-66.2)	<i>n</i> -6 long-chain PUFA	15.0±2.5	15.0±2.5	0.78
<i>n</i> -3 PUFA (mg/day)	729.4 (258.7-2066.4)	665.4 (237.7-1907.2)	<i>n</i> -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
<i>n</i> -3 long-chain PUFA (mg/day)	208.3 (24.5-1129.2)	196.8 (21.9-1037.9)	<i>n</i> -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 <i>cis</i> MUFA^c							

Mean Intake \pm SD (g/d)	13.76 \pm 2.63	19.77 \pm 1.39	24.65 \pm 1.47	30.64 \pm 2.11	44.32 \pm 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 (18:1n-9)							
Mean Intake \pm SD (g/d)	12.72 \pm 2.44	18.34 \pm 1.32	23.02 \pm 1.41	28.78 \pm 2.01	42.04 \pm 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 \pm SD (mg/d)	6.00 \pm 3.00	15.00 \pm 3.00	29.00 \pm 5.00	52.00 \pm 8.00	120.00 \pm 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 \pm SD (g/d)	0.30 \pm 0.14	0.74 \pm 0.12	1.21 \pm 0.15	1.93 \pm 0.28	4.18 \pm 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 (18:1n-9/12)							

Mean Intake \pm SD (g/d)	0.27 \pm 0.13	0.69 \pm 0.12	1.14 \pm 0.15	1.85 \pm 0.28	4.11 \pm 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 \pm SD (g/d)	6.51 \pm 1.17	9.21 \pm 0.63	11.42 \pm 0.67	14.17 \pm 0.97	20.69 \pm 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 (18:2n-6)							
Mean Intake \pm SD (g/d)	6.48 \pm 1.16	9.17 \pm 0.63	11.38 \pm 0.67	14.13 \pm 0.97	20.64 \pm 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 \pm SD (mg/d)	8.00 \pm 3.00	17.00 \pm 2.00	24.00 \pm 2.00	34.00 \pm 4.00	61.00 \pm 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 \pm SD (g/d)	0.29 \pm 0.08	0.49 \pm 0.05	0.67 \pm 0.06	0.93 \pm 0.10	1.70 \pm 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 (18:3n-3)							
Mean Intake \pm SD(g/d)	0.15 \pm 0.05	0.27 \pm 0.03	0.38 \pm 0.04	0.56 \pm 0.07	1.10 \pm 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 PUFAⁱ							
Mean Intake (mg/d)	40.00 \pm 21.00	110.00 \pm 21.00	200.00 \pm 27.00	340.00 \pm 60.00	920.00 \pm 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 \pm SD (g/d)	7.05 \pm 1.23	9.88 \pm 0.66	12.20 \pm 0.70	15.07 \pm 1.01	21.87 \pm 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 \pm SD	7.80 \pm 2.29	13.08 \pm 1.24	17.47 \pm 1.34	23.12 \pm 2.06	38.91 \pm 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; ^bOdd chain fatty acids included 15:0, 17:0; ^cTotal 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; ^dTotal trans ruminant fatty acids included 18:1n-7t, CLA; ^eTotal trans industrial fatty acids included 16:1n-9t, 18:1n-9t, 18:2n-6tt, 18:3n-3ttt; ^fTotal n-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4; ^gTotal long-chain n-6 PUFA included 20:2, 20:3, 20:4; ^hTotal n-3 PUFA included 18:3, 20:3, 20:5, 22:5, 22:6; ⁱTotal long-chain n-3 PUFA included 20:3, 20:5, 22:5, 22:6; ^jTotal 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 <i>cis</i> 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<i>n</i>-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 <i>trans</i> 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 <i>trans</i> 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<i>n</i>-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 <i>cis</i> <i>n</i>-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<i>n</i>-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 <i>n</i>-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 <i>cis n-3</i> 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 (18:3n-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 <i>n-3</i> 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 <i>cis</i> PUFAⁱ				
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 <i>n-6/n-3</i> 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

DI₁₆** (16:1n-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
DI₁₈ (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

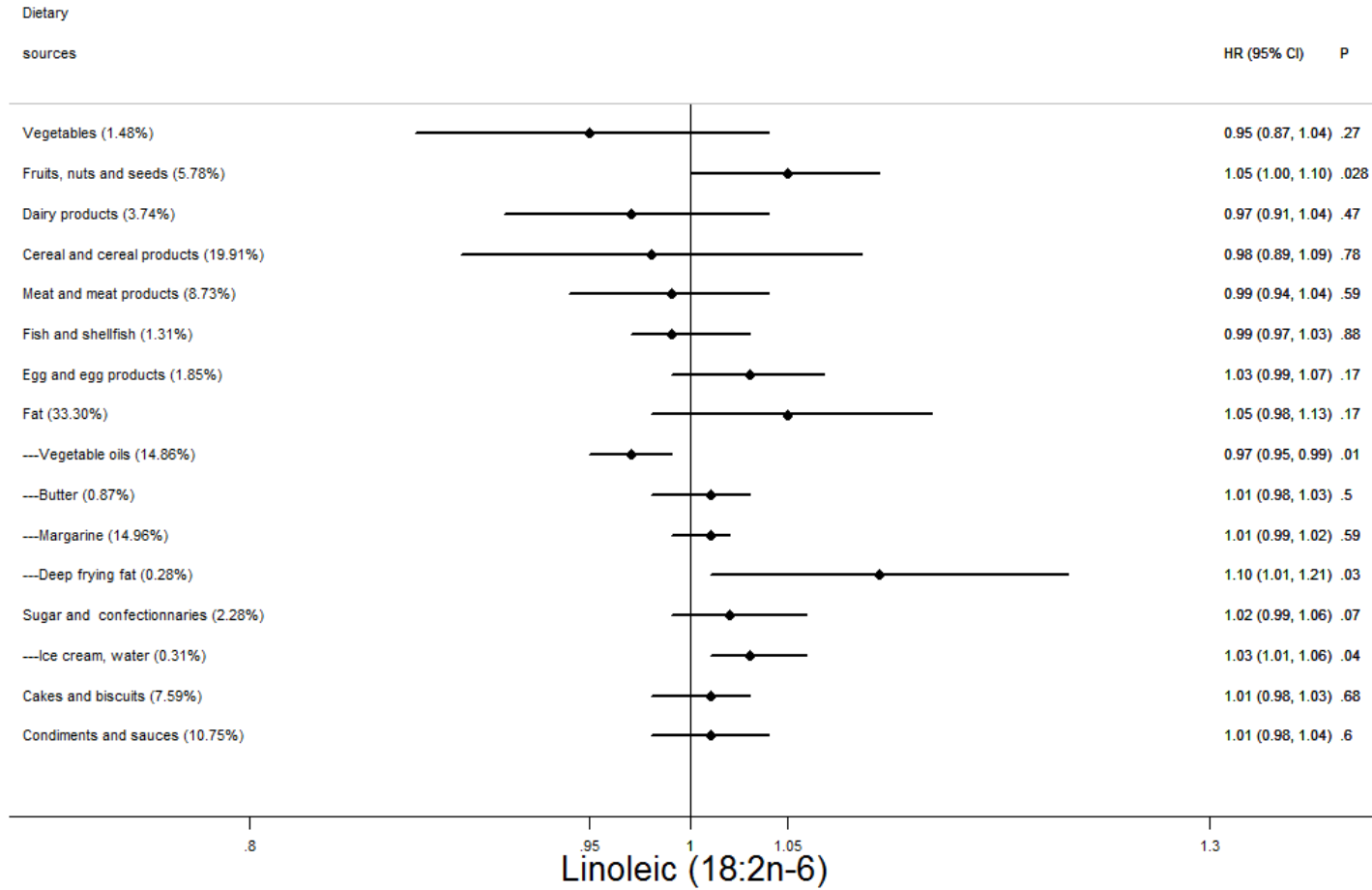
† P values < 0.05 are shown in boldface

* 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.

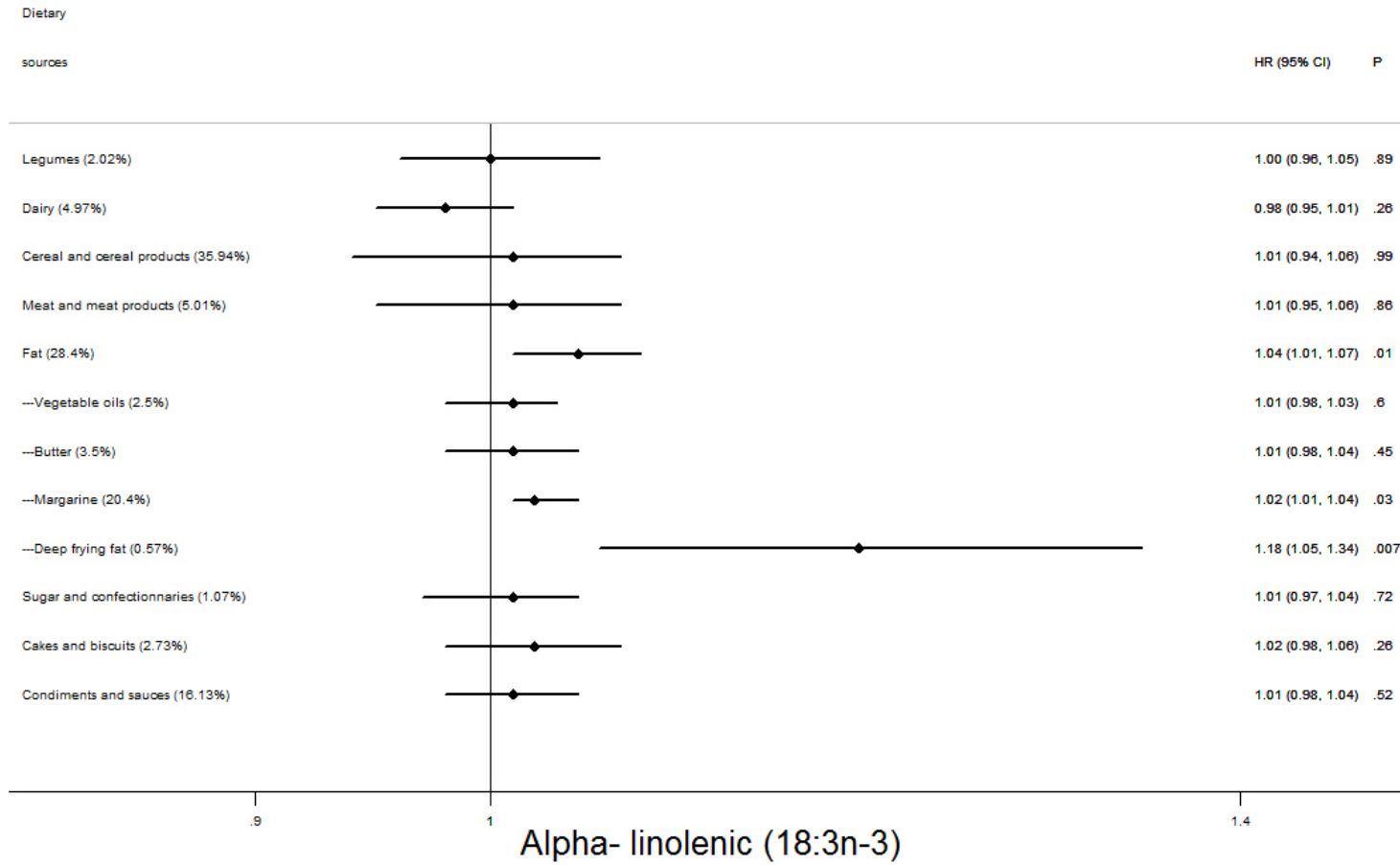
^aTotal SFA included 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; ^bTotal cis MUFA included 14:1, 15:1, 16:1n-7/n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1; ^cTotal trans ruminant fatty acids included 18:1n-7t, CLA; ^dTotal trans industrial fatty acids included 16:1n-7t/n-9t, 18:1n-12/n-9t, 18:2n-6tt, 18:2n-6tc, 18:3n-3ttt; ^eTotal n-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5; ^fTotal long-chain n-6 PUFA included 20:2, 20:3, 20:4, 22:4, 22:5; ^gTotal n-3 PUFA included 18:3, 18:4, 20:4, 20:5, 22:5, 22:6; ^hTotal long-chain n-3 PUFA included 20:4, 20:5, 22:5, 22:6; ⁱTotal cis PUFA included total n-6 PUFA and total n-3 PUFA

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.

Chapter 5. Dietary fatty acid and endometrial cancer risk within EPIC

The manuscript entitled “Dietary fatty acids and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition” is in preparation and will be submitted soon.

I will be the first author of this article.

My roles in the development of this study and the production of the manuscript were to: participate to the analytical strategy, conduct all statistical analyses and write the manuscript.

The preliminary form of this article is as following:

Abstract

Introduction. Fatty acids (FA) have been postulated to impact important risk factors for endometrial cancer (EC) such as obesity, estrogens and inflammation but few epidemiological studies are available. The associations between dietary FA and EC risk were investigated in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Methods. This study includes 1,886 incident EC cases and 297,432 non-cases. Multivariable Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of EC across quintiles (Q) of individual FA estimated from various food groups quantified through food frequency questionnaires in the entire EPIC cohort. The false discovery rate (q-values) was computed to control for multiple testing.

Results. A negative trend was found between n-6 γ -linolenic acid intake and EC risk (HR comparing 5th with 1st quintile_{Q5-Q1}=0.77, 95% CI=0.64; 0.92, p_{trend} =0.01, q-value=0.15). The overall inverse association between γ -linolenic acid and EC risk was mainly driven by its vegetable sources (HR=0.94, 95%CI= (0.90-0.98), p =0.01)) but not from animal sources (HR= 1.02, 95%CI = (0.94; 1.10), p =0.62). Furthermore, an inverse association was also found between n-3 α -linolenic acid from vegetable sources and EC risk (HR= 0.93, 95%CI = (0.87; 0.99), p =0.04) but not from animal sources (HR= 1.02, 95%CI = (0.95; 1.10), p =0.64). No significant association was reported between any other FA (individual or grouping) and EC risk.

Conclusion. Our results showed a significant inverse association between γ -linolenic acid and α -linoleic acid from plant sources but not from animal sources and EC risk. The dietary sources of fatty acids should be taken into account in epidemiological studies.

5.1. Introduction

382,069 new cases and 89,929 deaths from EC were identified in 2018 worldwide (9). In Europe, EC is the fourth most common cancer and the sixth most common cause of cancer death in women (9). As the incidence of EC cancer is rising worldwide, prevention strategies are urgently needed; however, few preventable factors have been identified (130). The evidence of an association between EC risk and specific dietary components is limited (52). A review on lifestyle factors and EC reported that diet and exercise can modulate the risk of developing EC (137). A review on diet and women cancers at menopause reported that sustained estrogen exposure has been associated with a higher risk of hormone-related cancers including EC and thus high-fat and meat diets have been linked with an increased risk (138). Limiting energy-dense foods rich in carbohydrates reduce metabolic abnormalities (such as obesity and insulin resistance) that promote endometrial pathology (52). A modest inverse association was also reported between vegetable consumption and EC risk (139).

In vivo and *in vitro* studies suggested that several components of diet, especially lipids including saturated FA (SFA), unsaturated FA, and cholesterol intake might influence the proliferation of EC cells by modulating the production, metabolism, and excretion of endogenous hormones (57, 58, 107, 108). Another possible hypothesis for the association between some FA and EC (66) is through inflammation as it is believed to play a central role in many of the chronic diseases including EC. As a matter of fact, some experimental studies suggested that inflammatory processes play a central role in the regulation of endometrial mucosa growth and shedding during the menstrual cycle (140) as well as in endometrial repair following menstruation (141). Hence, the association between these FA and EC risk might be mediated by inflammatory factors.

A systematic meta-analysis by the World Cancer Research Funds (WCRF) concluded that there was “limited” evidence for a link between total fat, saturated/animal fat and EC (52). Data from the EPIC and the Nurses' Health Study (NHS) and NHSII reported a higher risk of EC associated with a higher intake of total fat and monounsaturated fat (110). Data from a meta-analysis suggested a lack of association between total dietary fat intake and EC risk (111). Results from another meta-analysis of 7 cohorts and 14 case-control studies suggested that higher MUFA intake was associated with lower EC risk; total fat and saturated fat intake were associated with a higher risk of EC in the case-control studies of this meta-analysis while no significant association was suggested with PUFA and linoleic acid (112). Another meta-analysis of 8 case-control and 4 cohort studies suggested that intake of n-3 PUFA may be inversely associated with EC risk (113).

As results from epidemiological studies in this field are heterogeneous, and because of a lack of information on EC subtypes and the types of foods that drive these associations, the aims of this present study were:

- 3- To prospectively investigate the association between FA intake and EC risk, overall and by different levels of stratifications.
- 4- Determine the dietary sources of FA with a distinction between animal, vegetable and industrial food sources and EC risk in the EPIC study.

5.2. Materials & Methods

Study design

Among 299,455 eligible women, 2,023 malignant EC were identified after a mean follow-up of 8.8 years. Cases were censored because of the tumor morphology (n =73), or if they did not complete a lifestyle or dietary questionnaire (n = 26), or were classified in the top or bottom 1% of energy intake to energy requirement (n=38), leaving 1,886 EC incident cases and for the current analysis. Cancer end point data is based on the latest round of follow-up received from the EPIC centers and centralized at IARC between 2014-2016. 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).

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. The in-depth process for compiling this ENDB database was described in detail elsewhere (121, 132) and was used as a basis to match the EPIC data with FA isomers, using the National Nutrient Database for Standard Reference of the United States (NNDsr; further referred to as USDA table) (133). To date, most of the national food composition databases of the ten respective EPIC countries do not contain nutritional values for specific FA isomers. Therefore, specific foods and recipes that were not included in the USDA were decomposed in ingredients which were available in the USDA table and amounts of FA were obtained through this extra USDA matching. Groupings of FA were defined as: saturated fatty acids (SFA) (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), monounsaturated fatty acids (MUFA) (16:1n-7, 16:1n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1), n-6 polyunsaturated fatty acids (PUFA) (18:2, 18:3, 20:2, 20:3, 20:4) and n-3 PUFA (18:3, 20:3, 20:5, 22:5, 22:6), long-chain n-6 (20:2, 20:3, 20:4) long-chain n-3 PUFA (20:3, 20:5, 22:5, 22:6), ruminant *trans* fatty acids (rTFA) (18:1n-7, CLA), and industrial *trans* fatty acids (iTFA) (16:1n-9, 18:1n-9, 18:2n-6, 18:3n-3).

Statistical analysis

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 HR and 95% CI for the association between dietary FA and EC risk. Intakes of FA were divided into quintiles based on their distribution in all cohort participants at baseline, setting women in the lowest category of FA 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 BMI (continuous), number of full term pregnancies (number of live born and/or still born children; 0, 1-2, 3-4; >4; missing), smoking status, oral contraceptive or HRT use (never or ever), menopausal status at enrolment (premenopausal; postmenopausal; perimenopausal/unknown menopause), age at menarche (continuous) and total energy intake (continuous). Additional potential confounders including history/duration of breastfeeding, 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 FA for each other did not modify the risk estimates (data not shown). Tests for trend were computed using the quintile specific median of each FA.

Due to the number of tests performed, q-values were calculated using the false discovery rate of the Benjamini-Hochberg procedure (142).

Additionally, associations between individual FA intakes (as continuous variables) and EC risk were investigated by their dietary sources grouping vegetable sources and animal sources. The percentage of contribution was calculated for both food sources based on the mean daily intake reported in the questionnaire.

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

5.3. Results

Compared to the non-cases, the EC 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 (**Table 3**).

A negative trend was found between n-6 γ -linolenic acid intake and EC risk (HR comparing 5th with 1st quintile_{Q5-Q1}=0.77, 95% CI=0.64; 0.92, p_{trend} =0.01, q-value=0.15). The overall inverse association between γ -linolenic acid and EC risk was mainly driven by its vegetable sources (HR=0.94, 95%CI= (0.90-0.98), p =0.01) (**Figure 16**). Another inverse association was also found between n-3 α -linolenic acid from vegetable sources and EC risk (HR= 0.93, 95%CI = (0.87; 0.99), p =0.04) (**Figure 17**).

No other significant association were reported between vegetable or animal sources of other FA and EC risk (**Table 4**).

In addition, no significant association was reported between TFA from industrial sources and EC risk (**Table 4**).

Finally, the association between FA and EC did not vary according to histological subtypes of EC (data not shown). Stratified analysis by BMI, parity, menopausal status lag time did not show any substantial differences in the risk estimates (data not shown). All p for heterogeneity >0.05 .

5.4. Discussion

We found evidence of an inverse association between n-6 γ -linolenic acid and n-3 α -linolenic acid and EC risk, mainly driven by their vegetable sources. These associations did not vary according to histological subtypes of EC.

Besides γ -linolenic acid and α -linolenic acid from vegetable sources, no significant association between any other dietary FA and EC was reported in this study. Our results are aligned with results from the NHS and NHS II studies (110) but not with findings from a previous EPIC study which reported an inverse association between total fat, total MUFA and EC (110). This is probably due to the fact that we looked at the associations between individual FA and EC risk rather than groupings of FA. As a matter of fact, FA can have different effects, even within the same family, and considering FA at the aggregated level may mask or obscure some associations.

iTFA consumption is associated with increased all-cause mortality (143) and the WHO encourages the elimination of these FA from the diet (129). Few epidemiological data are available on the association between iTFA and cancer risk. However, and in agreement with other studies (112, 113), we did not report any significant association between iTFA and EC. Contrary to the positive association that we reported with breast and OC development in the EPIC cohort (76, 144), this present study suggest that iTFA from industrial processes are not associated with EC development.

An overall inverse association was reported between γ -linolenic acid and EC risk mainly driven by its vegetable sources. In addition, an inverse association was also found between n-3 α -linolenic acid from vegetable sources and EC risk while no significant association was found for animal sources. To our knowledge, no previous studies have investigated the associations between animal and plant sources of FA and EC risk. Only one recent study in the NHS and HPFS (Health Professionals Follow-Up Study) suggested that higher intake of MUFA from plant sources was associated with lower over-all mortality including cardiovascular and cancer mortality (145). Our data suggest that vegetable sources of γ -linolenic acid and α -linolenic acid may exert a protective effect on EC risk. Indeed, total vegetable, cruciferous vegetable and particularly non-starchy vegetables in addition to total fruit may protect from EC (139). This protective role might involve the modulation of steroid hormone concentrations and metabolism, activation of antioxidant mechanisms, modulation of detoxification enzymes, and stimulation of the immune system (146).

No significant association was found between n-6 and n-3 PUFA and EC risk in this study. However, it is known that these two families play a significant role in health and disease including cancer. Their effect might be mediated by generating potent modulatory molecules for inflammatory responses, including eicosanoids (prostaglandins, and leukotrienes), and cytokines (interleukins) and by affecting the gene expression of various bioactive molecules. γ -Linolenic acid (18:3n-6) (derived from linoleic acid, an essential FA by Δ 6-desaturase) can be elongated by the enzyme elongase 5 to dihomo- γ -linolenic acid (20:3n-6; DGLA)(147). Then, DGLA undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids (prostaglandins of series 1 and leukotrienes of series 3) (148). Using the same series of enzymes as used to metabolize n-6 PUFAs, n-3 α -linolenic acid is converted into long-chain FA (LC-PUFA): eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3;DHA). EPA and DHA are also found in oily fish and fish

supplements. These FA are capable, in one part, of inhibiting many aspects of inflammation including leucocyte chemotaxis, adhesion molecule expression and leucocyte–endothelial adhesive interactions, production of eicosanoids like prostaglandins and leukotrienes from the n-6 arachidonic acid and production of pro-inflammatory cytokines. In a second part, EPA and DHA give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins and maresins (147).

Regarding EC, inflammation has been related to this cancer in several cohort and case-control studies. (149-151). Suggested to being anti-inflammatory (as described above), LC-PUFA (EPA and DHA) could potentially reduce EC risk (152). However, epidemiological results in this field are inconclusive. One Japanese case-control study reported a lower risk of EC in association with higher fish consumption (153) whereas several other case-control and cohort studies reported no significant association (116, 154, 155). Similarly, our data reported no significant association between n-3 LC-PUFA and EC risk. Further replications and clarifications are needed to find a potential association between n-3 LC-PUFA, fish intake and EC risk.

Our study has several strengths including its prospective design, and a very large number of incident EC cases. Additionally, we were able to separate *n-6* and *n-3 cis* PUFA isomers. The major limitation of the study is the single collection of questionnaires and blood samples at baseline.

5.5. Conclusion

Our findings in the EPIC study suggested that essential n-6 and n-3 PUFA may exert a protective effect on EC development, mainly driven by vegetable sources suggesting that the dietary source of FA (animal versus vegetal) is determinant when investigating the association

between FA and cancer risk. Adherence to a diet rich in vegetables and fruits might be recommended to reduce EC risk.

5.6. Funding

This work was undertaken during the tenure of my doctoral Fellowship supported by the Fondation ARC pour la recherche sur le cancer (ARC 2019-DOC4).

5.7. Tables and figures

Table 3. Characteristics of the study population

EPIC-wide study	Endometrial cancer cases		Non cases*	
	n=1,886		n=297,432	
N= 299,318				
Anatomical subtypes, number, (%**)				
Endometrioid	984 (52.2)		-	
Serous Clear cell	84 (4.5)		-	
Follow-up characteristics				
Mean±SD**				
Age at recruitment, years	54.9±7.5		50.2±9.9	
Age at diagnosis, years	63.7±8.1		-	
Follow-up, years	8.8±4.7		14.0±3.8	
Anthropometry Mean±SD**				
Weight, kg	70.9±13.9		65.3±11.6	
Height, cm	162.2±6.6		162.3±6.7	
BMI, kg.m ⁻²	26.9±5.3		24.8±4.4	
Obese (BMI ≥30 kg/m ²), %**	24.2		12.1	
Reproductive and hormone factors				
Number of full term pregnancies#	1.9±1.2		1.9±1.2	
Nulliparous, %	16.4		15.6	
Ever use Oral contraceptives, %**				
Never	58.6		40.2	
Ever	41.4		59.7	
Ever use hormone replacement therapy##, %**				
Never	66.3		76.9	
Ever	33.7		23.1	
Ever breastfed#, %**				
No	28.7		27.9	
Yes	71.3		72.1	
Menopausal Status, %**				

Premenopausal	20.5	38.2
Post-menopausal	59.5	43.8
Perimenopausal	19.6	17.7
Age at menopause##	50.9±4.1	49.3±4.4
Socio-economic status and lifestyle		
Total energy intake, Kcal/day	1949.5±539.8	1993.1±546.1
Alcohol intake, %**		
None	18.5	16.4
<5g/day	32.7	31.9
5 to <14.9 g/day	28.2	30.1
15.0 to <29.9 g/day	12.7	13.2
≥29.9 g/day	7.3	7.7
Education status, %		
None and primary school	33.8	27.8
Technical or professional and secondary school	44.1	45.0
Higher education	17.8	23.6
Physical activity status, %**		
Inactive	14.2	15.2
Moderately inactive	34.5	35.9
Moderately active	43.4	40.7
Active	7.9	8.2
Smoking status, %**		
Never	62.7	56.8
Former	21.7	22.9
Current	15.5	20.2
Fruits and vegetables intake, (g/day) Median (95% CI)**		
Fruits and vegetables	424.1(150.7-918.7)	420.4 (142.5-955.9)
Fatty acid intake### (g/day or mg/day) Median (95% CI)**		
SFA (g/day)	23.7(11.2-45.8)	24.9 (11.7-48.3)
<i>Cis</i> MUFA (g/day)	23.4 (11.3-45.2)	24.7 (12.1-48.5)
rTFA (mg/day)	22.9 (3.1-116.4)	27.9 (4.3-134.5)

iTFA (g/day)	1.2 (0.1-5.3)	1.2 (0.2-4.9)
n-6 PUFA (g/day)	11.0 (5.4-21.4)	11.4 (5.8-22.5)
n-6 linoleic acid (g/day)	10.9 (5.3-21.3)	11.38 (5.7-22.4)
n-6 γ -Linolenic acid (mg/day)	6.9 (1.9-22.5)	7.3 (1.8-24.3)
n-6 long-chain PUFA (mg/day)	23.7 (7.4-66.7)	24.2 (5.5-66.0)
n-3 PUFA (mg/day)	701.5 (254.0-1945.3)	667.2 (237.5-1913.2)
n-3 α -linolenic acid (mg/day)	379.4 (116.7-1263.6)	382.2 (117.5-1251.9)
n-3 long-chain PUFA (mg/day)	229.1 (28.8-1156.8)	198.1 (21.7-1051.9)

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

***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 chapter 4

Table 4. Association of estimated dietary intakes of fatty acids with endometrial cancer risk in the EPIC cohort

	Q1	Q2	Q3	Q4	Q5	p trend [†]	q trend [§]
	Reference						
Total SFA^a							
Mean Intake ±SD (g/d)	13.49±2.79	19.88±1.47	25.01±1.53	31.12±2.11	44.59±9.16		
Cases/non-cases (n)	460/59,404	389/59,475	379/59,484	338/59,526	320/59,543		
HR (95% CI) ^{#*}	1.00	0.89 (0.77;1.03)	0.91 (0.77;1.06)	0.87(0.73;1.05)	0.94 (0.75;1.18)	0.45	0.86
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	22.49±4.15		
Cases/non-cases (n)	442/59,422	402/59,462	396/59,467	335/59,529	311/59,552		
HR (95% CI) [*]	1.00	0.95 (0.82;1.10)	0.96 (0.81;1.12)	0.88(0.73;1.06)	0.90 (0.71;1.14)	0.25	0.86
Total cis-MUFA^b							
Mean Intake ±SD (g/d)	13.79±2.64	19.86±1.40	24.77±1.48	30.82±2.13	44.61±9.49		
Cases/non-cases (n)	437/59,427	404/59,460	403/59,460	359/59,505	283/59,580		
HR (95% CI) [*]	1.00	1.02 (0.88;1.18)	1.09 (0.93;1.29)	1.06(0.87;1.29)	0.99 (0.77;1.28)	0.72	0.96
Oleic acid (18:1n-9)							
Mean Intake ±SD (g/d)	12.72±2.45	18.45±1.33	23.14±1.42	28.96±2.04	42.32±9.26		
Cases/non-cases (n)	448/59,417	402/59,461	391/59,472	365/59,499	280/59,583		
HR (95% CI) [*]	1.00	0.99 (0.86;1.15)	1.04 (0.88;1.23)	1.06(0.87;1.29)	0.97(0.75;1.25)	0.74	0.96
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)	493/59,372	362/59,502	386/59,476	330/59,534	315/59,548		
HR (95% CI) [*]	1.00	0.95 (0.81;1.11)	1.10 (0.93;1.29)	1.02(0.85;1.22)	1.13 (0.93;1.38)	0.22	0.86

Total industrial trans fatty acids^e

Mean Intake \pm SD (g/d)	0.30 \pm 0.14	0.73 \pm 0.12	1.20 \pm 0.15	1.93 \pm 0.28	4.19 \pm 1.68		
Cases/non-cases (n)	358/59,506	356/59,508	369/59,494	391/59,473	412/59,451		
HR (95% CI)*	1.00	1.12 (0.95;1.32)	1.08 (0.91;1.29)	1.08(0.90;1.30)	1.05 (0.86;1.27)	0.92	0.98

Elaidic acid (18:1n-9/12)

Mean Intake \pm SD (g/d)	0.27 \pm 0.13	0.69 \pm 0.12	1.13 \pm 0.15	1.85 \pm 0.28	4.13 \pm 1.68		
Cases/non cases (n)	357/59,507	353/59,511	364/59,499	398/59,466	414/59,449		
HR (95% CI)*	1.00	1.12 (0.95;1.33)	1.09 (0.91;1.31)	1.11(0.92;1.34)	1.06 (0.87; 1.30)	0.77	0.96

Total cis n-6 PUFA^f

Mean Intake \pm SD (g/d)	6.51 \pm 1.17	9.21 \pm 0.63	11.43 \pm 0.67	14.20 \pm 0.98	20.76 \pm 4.83		
Cases/non-cases (n)	468/59,396	360/59,504	360/59,503	389/59,475	309/59,554		
HR (95% CI)*	1.00	0.87 (0.75;1.01)	0.87 (0.74;1.02)	1.01(0.85;1.20)	0.83 (0.67;1.01)	0.43	0.86

Linoleic acid (18:2n-6)

Mean Intake \pm SD (g/d)	6.48 \pm 1.16	9.18 \pm 0.63	11.40 \pm 0.67	14.15 \pm 0.97	20.70 \pm 4.80		
Cases/non-cases (n)	468/59,396	358/59,506	364/59,499	386/59,478	310/59,553		
HR (95% CI)*	1.00	0.86 (0.75;1.00)	0.88 (0.75;1.03)	1.00(0.85;1.19)	0.83 (0.68;1.02)	0.46	0.86

 γ -linolenic acid (18:3n-6)

Mean Intake \pm SD (mg/d)	2.48 \pm 0.86	4.92 \pm 0.66	7.38 \pm 0.78	10.91 \pm .38	21.79 \pm 9.58		
Cases/non-cases (n)	388/59,495	393/59,454	414/59,461	378/59,474	313/59,548		
HR (95% CI)*	1.00	0.92 (0.80;1.07)	0.97(0.83;1.13)	0.91(0.78;1.08)	0.77(0.64;0.92)	0.01	0.15

Total long-chain n-6 PUFA^g

Mean Intake \pm SD (mg/d)	8.00 \pm 3.00	17.00 \pm 2.00	24.00 \pm 2.00	34.00 \pm 4.00	61.00 \pm 24.00		
Cases/non-cases (n)	356/59,510	396/59,471	386/59,477	390/59,473	358/59,501		
HR (95% CI)*	1.00	0.94 (0.81;1.10)	0.96 (0.81;1.12)	0.99(0.84;1.17)	0.93 (0.77;1.11)	0.65	0.96

Total cis n-3 PUFA^b

Mean Intake \pm SD (g/d)	0.29 \pm 0.08	0.49 \pm 0.05	0.67 \pm 0.06	0.93 \pm 0.10	1.70 \pm 0.61		
Cases/non-cases (n)	350/59,514	372/59,492	341/59,522	395/59,469	428/59,435		
HR (95% CI)*	1.00	1.00 (0.85;1.17)	0.91 (0.77;1.08)	0.97(0.81;1.15)	0.91 (0.75;1.10)	0.33	0.86

 α -linolenic acid (18:3n-3)

Mean Intake \pm SD(g/d)	0.15 \pm 0.05	0.27 \pm 0.03	0.38 \pm 0.04	0.56 \pm 0.07	1.10 \pm 0.44		
Cases/non-cases (n)	367/59,497	405/59,461	379/59,482	350/59,515	385/59,477		
HR (95% CI)*	1.00	1.08 (0.93;1.26)	1.05 (0.89;1.23)	0.96(0.81;1.14)	0.94 (0.78;1.14)	0.27	0.86

Total long-chain n-3 PUFAⁱ

Mean Intake (mg/d)	40.00 \pm 21.00	115.00 \pm 21.00	198.00 \pm 27.00	338.00 \pm 61.00	933.00 \pm 609.00		
Cases/non-cases (n)	333/59,531	320/59,545	363/59,499	401/59,463	469/59,394		
HR (95% CI)*	1.00	0.91 (0.77;1.07)	0.95 (0.80;1.12)	0.95(0.80;1.13)	0.95 (0.79;1.15)	0.84	0.96

Ratio n-6/n-3 PUFA

Mean Intake \pm SD	7.76 \pm 2.29	13.06 \pm 1.24	17.47 \pm 1.34	23.16 \pm 2.07	39.06 \pm 28.95		
Cases/non-cases (n)	491/59,373	419/59,445	334/59,529	334/59,530	308/59,555		
HR (95% CI)*	1.00	1.11 (0.96;1.28)	0.96 (0.81;1.13)	1.03(0.86;1.22)	1.04 (0.86;1.24)	0.98	0.98

HR = hazard ratio; CI = confidence interval

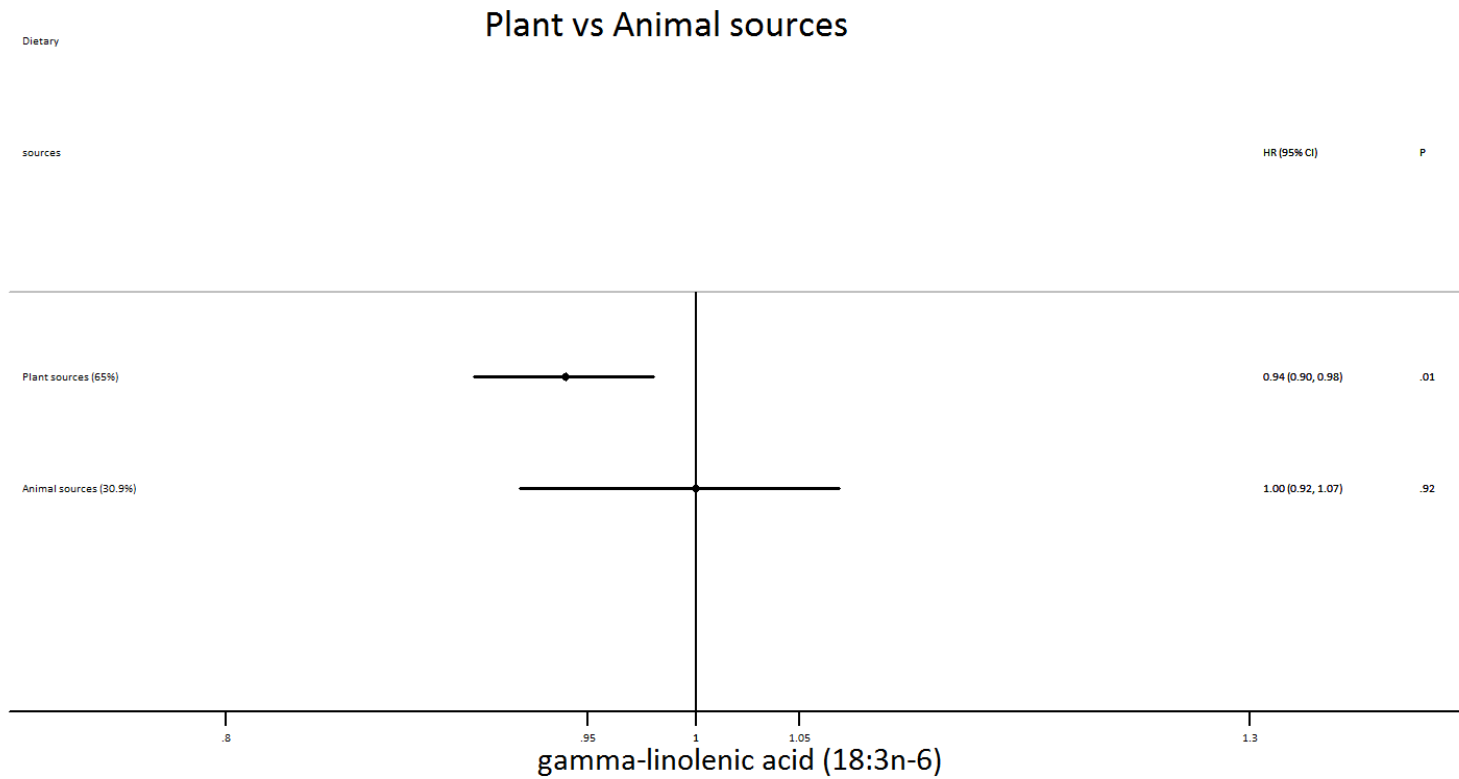
† P or q values < 0.05 are shown in boldface type

§ Value for FDR (False Discovery Rate) correction

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

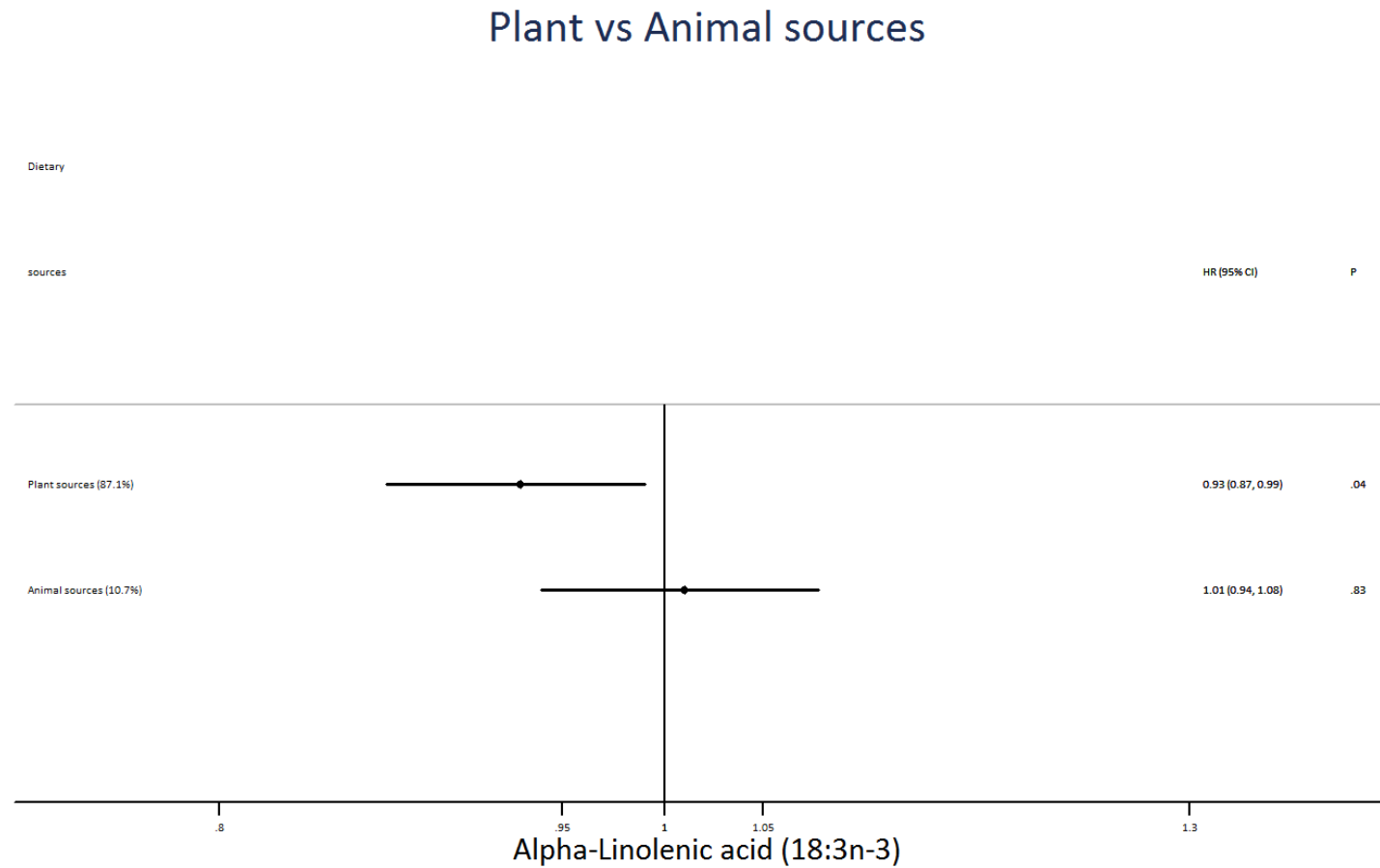
^aTotal SFA included 4:0,6:0, 8:0,10:0,12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; ^bOdd chain fatty acids included 15:0, 17:0; ^cTotal cis MUFA included 16:1n-7/n-9, 17:1, 18:1n-5, 18:1n-7, 18:1n-9, 20:1, 22:1, 24:1; ^dTotal trans ruminant fatty acids included 18:1n-7t, CLA; ^eTotal trans industrial fatty acids included 16:1n-9t, 18:1n-9t, 18:2n-6tt, 18:3n-3ttt; ^fTotal n-6 PUFA included 18:2, 18:3, 20:2, 20:3, 20:4; ^gTotal long-chain n-6 PUFA included 20:2, 20:3, 20:4; ^hTotal n-3 PUFA included 18:3, 20:3, 20:5, 22:5, 22:6; ⁱTotal long-chain n-3 PUFA included 20:3, 20:5, 22:5, 22:6; ^jTotal cis-PUFA included total n-6PUFA and total n-3 PUFA.

Figure 14. Associations between plant and animal sources of gamma-linolenic acid and endometrial cancer risk



The percentage of contribution next to the food sources 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 source to the gamma-linolenic acid intake. Contribution of the plant sources to γ -linolenic acid = 65.0% vs animal sources =30.9%. HR = Hazard Ratio; CI = confidence interval. The multivariable model was adjusted for BMI (continuous), number of full term pregnancies (number of live born and/or still born children; 0, 1-2, 3-4; >4; missing), smoking status, oral contraceptive or HRT use (never or ever), menopausal status at enrolment (premenopausal; postmenopausal; perimenopausal/unknown menopause), age at menarche (continuous) and total energy intake (continuous).

Figure 15. Associations between plant and animal sources of alpha-linolenic acid and endometrial cancer risk



The percentage of contribution next to the food sources 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 source to the alpha-linolenic acid intake. Contribution of the plant sources to α -linolenic acid = 87.1% vs animal sources = 10.7%. HR = Hazard Ratio; CI = confidence interval. The multivariable model was adjusted for BMI (continuous), number of full term pregnancies (number of live born and/or still born children; 0, 1-2, 3-4; >4; missing), smoking status, oral contraceptive or HRT use (never or ever), menopausal status at enrolment (premenopausal; postmenopausal; perimenopausal/unknown menopause), age at menarche (continuous) and total energy intake (continuous).

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***Chapter 6. Association between Serum
Phospholipid Fatty Acid Levels and Adiposity
among Lebanese Adults: A Cross-Sectional Study***

In this study, in collaboration with AUB, we characterised of FA biomarkers in relation to indicators of obesity in a Lebanese population. This work represents the first this kind in the Middle East and North Africa region, providing timely a baseline in a country under nutritional transition.

6.1. Background & aims

Increases in obesity prevalence have been reported during the last decade in the Lebanese population, which is currently undergoing nutritional transition. Based on experimental data and biological plausibility, FAs have been postulated to impact obesity, but few epidemiological studies addressing this hypothesis have been conducted. Because of the limitations inherent to dietary questionnaires such as biases and errors that affect their accuracy, the use of highly specific biomarkers of FAs is a complementary approach to enable a better understanding of its impact on obesity. The measurement of plasma or serum PL-FAs offer the potential to capture specific biomarkers of past dietary intakes (weeks to months) of FAs that cannot be endogenously synthesized, along with biomarkers of endogenous synthesis. Building on this framework, the aim of this study was to determine the association between the levels of serum PL-FAs (as biomarkers of dietary exposure and of endogenous FA metabolism) and obesity indicators (BMI and waist) in adults constituting a cohort of 395 Lebanese residents in the Greater Beirut area.

6.2. Materials & Methods

6.2.1. Population study

This cross-sectional study is based on a cohort constituted of 501 Lebanese adults. The sample for this study was drawn for an earlier community-based survey of a representative sample of Lebanese adults living in Greater Beirut area (**Figure16**) selected using a multistage stratified probability sampling frame. The original survey aimed to examine exposure to bisphenol A. Among these adults, FFQ and anthropometric measures were provided. A total of 395 left-over serum samples of 129 men and 266 women were analyzed for FAs in serum

phospholipids. Pregnant women, patients on dialysis and other vulnerable group (mentally disabled patients) were excluded.

Figure 16: The map of Lebanon



6.2.2. Statistical analysis

Percentages of FAs were log-transformed and geometric means with 95% interval of confidence (IC) were reported for the analysis. FAs or desaturation indices were correlated with obesity indicators (BMI and waist) using Spearman test. Correlation adjustments were performed for age, menopausal status in women, physical activity, smoking status, education, alcohol consumption, energy intake, and analytical batch.

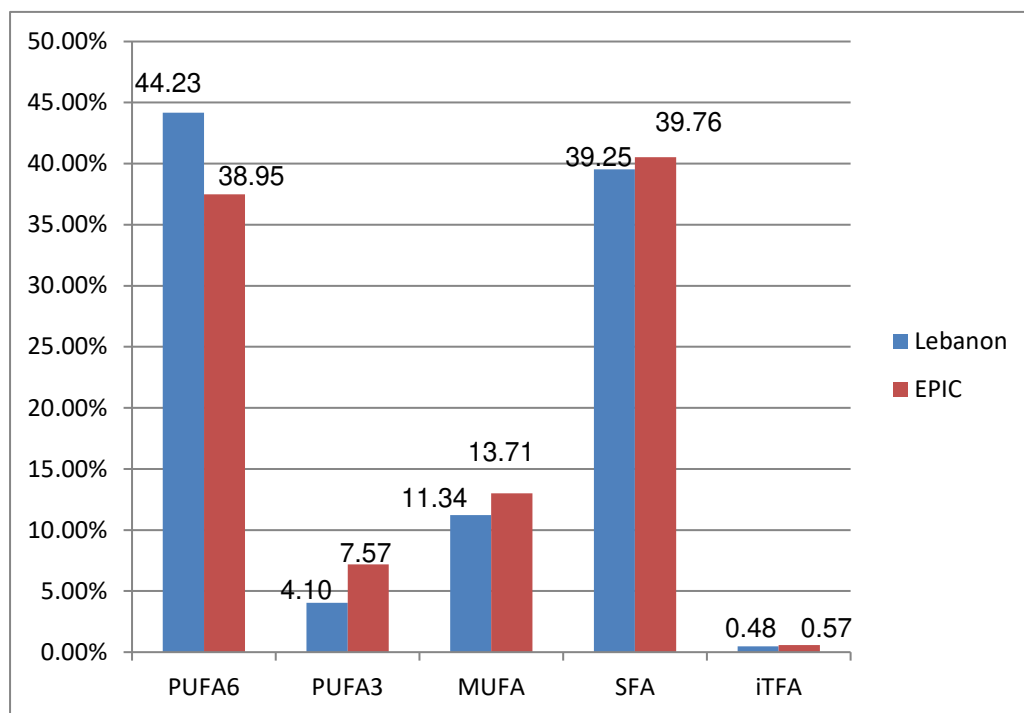
6.3. Results

In comparison with Mediterranean population of EPIC (indicate here the countries), serum PL-FAs profile in this Lebanese cohort is characterized by high levels of n-6 PUFA (44.23% in this Lebanese population vs 38.95% in Mediterranean regions in the EPIC study), low levels of n-3 PUFA (4.10 % vs 7.57%). Accordingly, the ratio of n-6/n-3 PUFA in the Lebanese population is much higher than in Mediterranean regions of EPIC (10.78 vs. 5.15) (**Figure 17**).

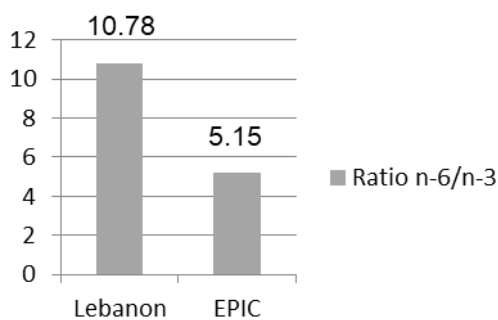
High blood levels of some SFA and *cis*-MUFA palmitoleic acid, likely to derive from dietary intake of SFA and increased endogenous lipogenesis, were positively correlated with adiposity (BMI and waist) in both men and women of this Lebanese population.

Inconsistent trends were found in this study between levels of total iTFAs, individual iTFA isomers, and BMI or waist circumference among women, albeit these correlations were weak. When we distinguished individual TFA isomers, we found differential correlations with BMI according to gender, with elaidic acid and *trans* linoleic acid showing significant inverse correlations in women, while *trans* isomers of α -linolenic acid showing a positive trend in men.

Figure 17. Percentages of fatty acids: Lebanese population vs Mediterranean populations of EPIC



Ratio n-6/n-3



Higher levels of n-6 PUFA and lower levels of n-3 PUFA in this Lebanese population compared to the levels of n-6 PUFA in Mediterranean regions of EPIC. Thus, the ratio of n-6/n-3 PUFA in the Lebanese population. Lower levels of MUFA in the Lebanese population. The levels of SFA and *trans* elaidic acid in this study population were found comparable to those reported in Mediterranean regions of EPIC.

6.4. Discussion

Total SFAs were significantly positively correlated with BMI and waist circumference in this study. Similar trends between total SFAs and BMI have been reported in other epidemiological studies with the same cross-sectional design, but also clinical trials and prospective designs (156-158). In addition, we found that palmitoleic acid and DI_{16} , as biomarkers of endogenous lipogenesis, were positively correlated with BMI, particularly in women. Our data further suggest that an increased endogenous synthesis of palmitoleic acid may increase adiposity. In agreement with our findings, some epidemiological studies have consistently reported a positive association between adipose tissue or circulating palmitoleic acid, DI_{16} and obesity (159-161). Further studies are needed to explore the causality of these associations.

Inconsistent trends were found in this study between levels of total iTFAs, individual iTFA isomers, and BMI or waist circumference. However, several epidemiological studies with prospective design, reported an increased risk of weight gain associated with high levels of iTFA (162, 163). Furthermore, data from experimental models suggested that iTFA may induce obesity. A long-term intervention study on primates reported an increase of body weight in animals receiving an iTFA diet compared to those receiving *cis*-fatty acids (164, 165). Another study showed that a diet high in *trans*-fat induces insulin resistance pathway and obesity (166). Further studies are needed to clarify the causality of these associations.

The consumption of *trans* fats has long been recognized as a great public health concern particularly for their association with NCDs and higher obesity rates. As a matter of fact, the WHO called to ban iTFA from diet by 2023 through the REPLACE initiative in order to reduce NCD risks, including obesity. Today, iTFA may have decreased in processed foods in some countries though not necessarily in certain vulnerable groups (126) or in LMICs. Lebanon is a

LMIC undergoing nutritional transition, so timely intervention to limit increases in iTFAs is needed (167).

6.5. Conclusion and perspectives

This study has highlighted important differences between the Lebanese and the European population living in Mediterranean regions. In addition, this work suggested that high blood levels of some SFA and an increased endogenous lipogenesis were correlated with increased adiposity in the Lebanese population. Reducing SFA (mainly derived from palm oil, coconut oil, cocoa and animal-derived fat (butter, meat – such as fatty tallow of beef, pork and lamb, processed meats) intake could potentially offer a public health strategy for reducing BMI.

Obesity is a well-characterized cancer risk factor, and Lebanon ranks highly worldwide in the incidence of several cancer types (168). Our characterization of FA biomarkers in relation to the increasing obesity incidence in the Lebanese population represents the first study of this kind in the Middle East and North Africa region and offers a timely framework that could provide baseline data and guide future studies aiming to address cancer prevention strategies in this region.

6.6. Scientific article

“Association between Serum Phospholipid Fatty Acid Levels and Adiposity among Lebanese Adults: A Cross-Sectional Study” by Yammine S. G. *et al.*, has been published in Nutrient on 25 September 2018. I am the first author of this publication.

My roles in the development of this study and the production of the manuscript were to: participate to the analytical strategy, conduct all statistical analyses, write the manuscript and revise it according to the reviewers' comments.

Article

Association between Serum Phospholipid Fatty Acid Levels and Adiposity among Lebanese Adults: A Cross-Sectional Study

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Abstract: There have been increases in the incidence of obesity in Lebanon over the past few decades. Fatty acid intake and metabolism have been postulated to influence obesity, but few epidemiological studies have been conducted. The aim of this study was to investigate the correlation between serum fatty acid levels and indicators of obesity in a cross-sectional study nested within a cohort of 501 Lebanese adults residing in Greater Beirut. A total of 395 available serum samples (129 men, 266 women) were profiled for phospholipid fatty acid composition. Spearman correlation coefficients adjusted for relevant confounders and corrected for multiple testing were calculated between serum fatty acids, desaturation indices, and indicators of adiposity (body mass index (BMI) and waist). BMI was significantly positively correlated with saturated fatty acids in men ($r = 0.40$, $p < 0.0001$, $q < 0.0001$) and women ($r = 0.33$, $p < 0.0001$, $q < 0.0001$). BMI was significantly positively correlated with monounsaturated fatty acid palmitoleic acid in women ($r = 0.15$, $p = 0.01$, $q = 0.03$). This study suggests that high blood levels of some saturated fatty acids and the monounsaturated fatty acid palmitoleic acid, likely derived from both dietary intakes of saturated fatty acids and endogenous lipogenesis, may have been associated with adiposity in the Lebanese population. The causality of these associations needs to be explored in experimental settings.

Keywords: nutrition; fatty acids; endogenous lipogenesis; obesity; epidemiology; low-to-middle income countries

1. Introduction

The global prevalence of overweight adults in the world population has markedly increased from 24.6% in 1980 to 39% in 2016 [1,2]. Over the same period, the prevalence of obesity has nearly tripled worldwide from 6.4% to 13.0% [1,2]. In many countries, these changes have impacted the incidence of major non-communicable diseases including heart diseases, type 2 diabetes, and cancer [3]. In the eastern Mediterranean region, obesity rates in the adult population have reached high levels, exceeding at times those reported from developed countries such as the USA and Europe [4,5], with roughly one fifth of the adults in the region considered as obese [6]. Moreover, this increase in obesity rates

has occurred in a short timeframe in the Middle East and is continuing to escalate [4,5]. In Lebanon, available data suggests that the prevalence of obesity increased significantly between the years 1997 and 2009 among adults aged 20 years and above (17.4% in year 1997 versus 28.2% in year 2009) [5].

The global expansion in obesity is predominantly attributed to changes in the obesogenic environment, characterized by (i) an upsurge in dietary energy intake, (ii) a higher consumption of added monosaccharides and of saturated and *trans* fatty acids, and (iii) an exceptional shift in energy expenditure patterns tilted towards a decrease in physical activity and an increase in sedentary behaviors [7]. Fat metabolism and dietary fatty acids have been postulated to affect obesity, estrogen levels, insulin resistance, and inflammation [8,9]. Several epidemiological studies have examined the relationship between dietary fatty acids estimated through dietary questionnaires and obesity, but the evidence remains inconclusive. Melanson et al. summarized interventional, prospective cohorts and cross-sectional studies investigating the associations between intakes of saturated acids (SFAs), monounsaturated acids (MUFAs), industrially-produced *trans* fatty acids (iTFAs), polyunsaturated fatty acids (PUFAs), and risk of obesity. The authors of this review reported that there is inconclusive evidence regarding the associations between the amount and types of fat intake and obesity. This review also underscored the inconsistencies in the literature and highlighted the limitations of dietary assessment methods as potential reasons underlying these inconsistencies [10]. In fact, whether collected using dietary recalls or records methodologies, dietary intake estimations have inherent biases and errors that affect their accuracy.

As a complementary tool to information based on dietary assessment methods, the measurement of serum or plasma fatty acids might provide a more objective estimation to enable a better understanding of their impact on obesity. Hence, some epidemiological studies based on the use of circulating fatty acids have consistently reported a positive association between adipose tissue or circulating palmitoleic acid, DI_{16} , and obesity [11–15]. Furthermore, a prospective study conducted within the European Prospective Investigation on Cancer (EPIC) reported an increased risk of weight gain during the follow-up associated with increasing levels of plasma phospholipid industrial *trans* elaidic acid [16], suggesting that iTFAs might increase adiposity. Further epidemiological studies based on biomarkers of fatty acids are needed to clarify the association between fatty acids and obesity.

Based on this set of data, we hypothesized that a high intake of iTFAs, along with an increased endogenous synthesis of MUFAs, may increase adiposity. Building on this framework, the aims of this study are to characterize the serum phospholipid profile in a cross-sectional study designed among Lebanese adults residing in the Greater Beirut area, and to determine the correlation between fatty acids, as biomarkers of dietary exposure and endogenous fatty acid metabolism, and obesity indicators.

2. Materials and Methods

2.1. Study Population and Recruitment

The target population constituted of Lebanese adults (>18 years) residing in the Greater Beirut area. The study sample for this study was drawn from an earlier community-based survey of a representative sample of Lebanese adults living in Greater Beirut area selected using a multistage stratified probability sampling frame. Details on the sampling used in this study are described elsewhere [17]. Pregnant women, patients on dialysis, and other vulnerable groups (mentally disabled patients) were excluded. Furthermore, given that the original survey aimed to examine exposure to bisphenol A (BPA) among adults residing in Beirut, participants working in plastic or other chemical companies were excluded as they may have been occupationally exposed to bisphenol A. Of the total 501 study participants, 395 participants consented to the use of their serum samples for future studies and hence were included in the current study.

2.2. Data Collection

In a face-to-face interview, trained interviewers completed a detailed data collection form for each subject. This questionnaire included information pertaining to the participants' medical history (all diseases that are associated with BPA and medications), diet through a food frequency questionnaire (FFQ) as well as lifestyle habits (smoking, alcohol, coffee, and physical activity), and socio-demographic information (age, gender, residence and previous travel, education, occupation, and income), physical exams (anthropometric data, weight, height, BMI (body mass index), waist circumference, and blood pressure), and a collection of urine and blood samples. Blood samples were collected after an overnight fast. The FFQ was an 80-item, semi-quantitative questionnaire, referring to subjects' dietary intake 12 months prior to the interview [17]. A tetrapolar single-frequency (330 μ A at 100 kHz) electrical bioimpedance analyzer was used to measure body composition. All interviews, physical examinations, and collection of biological samples were performed at the Nutrition and Food Sciences department, American University of Beirut (AUB).

2.3. Ethical Considerations

The protocol of the original survey was approved by the Institutional Review Board (IRB) at AUB. This study was approved by both the AUB IRB and the Ethical Committee of the International Agency for Research on Cancer.

2.4. Analysis of Serum Phospholipid Fatty Acids

For the purpose of this study, the 395 available blood samples were shipped to the International Agency for Research on Cancer (IARC) and stored at -80 °C until analyzed. As previously described [18], total lipids were extracted from serum samples, the phospholipid fraction was purified by adsorption chromatography, and Methyl-Prep II was used for the thansmethylation of fatty acids into fatty acid methyl esters. Fatty acid methyl esters were eluted on a gas chromatograph 7890A (Agilent Technologies, Santa Clara, CA, USA). Select for Fame capillary columns and specific for TFA separation were used for the separation of fatty acid methyl esters. Fatty acids are expressed as percent of total fatty acids and as absolute concentrations in serum (μ mol/liter) based on the quantity of L-A-phosphatidylcholine-dimyristoyl- d_{54} used as an internal standard. Overall (intra-batch and inter-batch) coefficients of variation (CVs) for fatty acids, which were calculated using two serum samples as quality controls added to each batch, ranged from 0.290% for large peaks, such as palmitic acid, to 9.340% for the smallest peaks, such as CLA. Overall CVs were 0.850 for saturated fatty acids, 0.291 for total monounsaturated fatty acids, 0.522 for industrial *trans* fatty acids, 0.312 for n-3 polyunsaturated fatty acids, and 0.974 for n-6 polyunsaturated fatty acids.

Using values for 60 individual fatty acids, the percentage and amounts of the following groups were calculated: SFAs, *cis*-MUFAs, ruminant *trans* fatty acids (rTFAs), iTFAs, *cis*-n-6 PUFAs, long-chain n-6 PUFAs, n-3 PUFAs, and long-chain n-3 PUFAs. We calculated the ratio of long-chain n-6/long-chain n-3 PUFAs. The desaturation indexes, as the ratio of product to substrate, either oleic acid to stearic acid (DI_{18}) or the ratio of palmitoleic acid to palmitic acid (DI_{16}), as biomarkers of endogenous lipogenesis, were also determined [19].

2.5. Statistical Analyses

The sociodemographic and lifestyle characteristics, as well as dietary intake of the study population, are represented in terms of frequencies for the categorical variables and means \pm standard deviations (SD) for the continuous variables. Fatty acids expressed in percentage of total fatty acids and in amounts were used for the statistical analysis. Fatty acid values were log-transformed and geometric means with 95% confidence interval (CI) were provided for the analysis. As first screening, fatty acids or desaturation indices were correlated with obesity indicators (BMI and waist circumference) using a partial Spearman test. Linear regression using the least squares method was

applied to test for potential non-linear associations between BMI and Waist with each of the fatty acids in turn. The model tested for both a linear and a squared term for the parameters. For the fatty acids, which were significantly correlated with BMI and Waist, we also assessed the assumptions of linearity using the linearity test, which showed that the analyzed correlations demonstrated a linear distribution; hence, we proceeded with statistical tests of linear correlation. Statistical analyses were also run in relation to percentage of body fat (data not shown). Adjustments were performed for the following factors: age (continuous variable), menopausal status in women (pre- and postmenopause), physical activity (total MET minutes/week), smoking status (non-smokers, current smokers, and ex-smokers), education (none, incomplete primary, complete primary, complete secondary, and complete high school), alcohol consumption (g/day), energy intake (kcal/day), and analytical batch. When considering the ratio of fatty acids (DI, n-6/n-3 PUFAs), fatty acids included in the ratio were further included in the statistical model. The coefficient of correlation (r) and the p -value were provided. Due to the number of tests performed, q -values were calculated by transforming the p -values for multiple comparisons using the false discovery rate of the Benjamini–Hochberg procedure [20]. Statistical analyses were performed using STATA version 14.1 (StataCorp, College Station, TX, USA) and R (R Foundation for Statistical Computing version 3.0.2, Vienna, Austria). A p -value ≤ 0.05 was used to indicate significance for all tests.

3. Results

3.1. Subjects Characteristics

General characteristics of the study participants are presented in Table 1, separately for men and women, the latter constituting approximately 2/3 of the participants. Overall, the studied population is characterized by a high BMI with a high percentage of obese subjects, a high percentage of smokers among men and women, with a significantly higher total energy intake in men than in women (Table 1).

Table 1. Baseline characteristics of the studied population.

	Mean ± SD or N (%) Total N = 395	Mean ± SD or N (%) Men N = 129	Mean ± SD or N (%) Women N = 266	p Value ^a
Age, years	44.5 ± 15.3	38.8 ± 16.3	47.3 ± 14.0	<0.0001
Anthropometry				
Weight, kg	75.2 ± 15.5	81.2 ± 15.5	72.2 ± 15.6	<0.0001
Height, cm	161.5 ± 9.8	172.2 ± 6.5	156.3 ± 6.4	<0.0001
Body-fat, kg	28.1 ± 11.4	22.6 ± 10.9	30.74 ± 10.7	<0.0001
BMI, kg/m ²	28.9 ± 5.7	27.4 ± 5.0	29.6 ± 5.9	0.0003
Percent body-fat, %	36.8 ± 10.9	26.7 ± 8.8	41.6 ± 8.1	<0.0001
Waist circumference, cm	94.5 ± 15.3	96.1 ± 12.7	93.8 ± 16.4	NS
BMI cut points, N (%)				0.01
Underweight and normoweight N (<25 kg/m ²)	103 (26.1%)	42 (32.6%)	61 (22.9%)	
Overweight (25–29.99 kg/m ²)	132 (33.4%)	48 (37.2%)	84 (31.6%)	
Obese (≥30 kg/m ²)	160 (40.5%)	39 (30.2%)	121 (45.5%)	
Percent body-fat cut points, N (%)				<0.0001
Normal				
≤25% for men		58 (44.9%)	51 (19.2%)	
≤35% for women				
Obese				
>25% for men		71 (55.1%)	215 (80.8%)	
>35% for women				
Waist circumference cut points, N (%)				<0.0001
Normal				
<94 cm for men		53 (41.1%)	50 (18.8%)	
<80 cm for women				
Increased risk of metabolic complications (94–102 cm) for men		39 (30.2%)	53 (19.9%)	
(80–88 cm) for women				
Substantially increased risk for metabolic complications				
>102 cm for men		37 (28.7%)	163 (91.3%)	
>88 cm for women				
Menopausal status, N (%)				
Pre-menopause			142 (53.4%)	
Post-menopause			124 (46.6%)	
Lifestyle factors				
Physical activity, total Mets/week	1731.9 ± 2129.7	1805.9 ± 2270.4	1696.0 ± 2061.5	NS
Smoking, N (% of current smokers)	258 (65.3%)	99 (76.7%)	159 (59.8%)	0.001
Nutritional factors				

Table 1. Cont.

	Mean ± SD or N (%) Total N = 395	Mean ± SD or N (%) Men N = 129	Mean ± SD or N (%) Women N = 266	p Value ^a
Energy intake, kcal/day	3361.2 ± 1969.9	4839.4 ± 2411.7	2644.3 ± 1174.9	<0.0001
Protein intake, g/day	109.9 ± 73.9	160.5 ± 83.4	85.4 ± 54.1	<0.0001
Percent of total energy intake	13.2	13.6	13.0	NS
Carbohydrate intake, g/day	415.9 ± 242.6	583.8 ± 301.1	334.5 ± 152.9	<0.0001
Percent of total energy intake	50.5	49.2	51.2	0.03
Total fat intake, g/day	138.7 ± 88.9	195.1 ± 109.4	111.4 ± 60.8	<0.0001
Percent of total energy intake	36.8	35.8	37.3	NS
Alcohol intake, g/day	7.4 ± 37.8	22.4 ± 63.8	0.18 ± 1.1	<0.0001
Percent of total energy intake	0.86	2.53	0.05	<0.0001

^a Independent-sample *t*-test or chi-square test. BMI: Body Mass Index; NS: non-significant; SD: standard deviation.

3.2. Serum Phospholipid Fatty Acid Composition

Serum phospholipid fatty acids, expressed as a percentage of total fatty acids, are indicated in Table 2, separately for men and for women. Individual fatty acids are grouped by family (SFAs, MUFAs, rTFAs, iTFAs, and n-6 and n-3 PUFAs) and by conformation (*trans* and *cis*).

Palmitic acid (16:0) and linoleic acid (18:2n-6*cis*) were the most abundant fatty acids in men and women in this population, accounting for the high percentages of total SFAs and total n-6 PUFAs, respectively (Table 2). The percentage of n-3 PUFAs was substantially lower than n-6 PUFAs, exhibiting a high ratio n-6/n-3 PUFA of 10.46 in men and 10.94 in women. Among TFA isomers, iTFAs represented 0.50% in men and 0.48% in women, while rTFAs represented 0.15% in men and in women. Total MUFAs, iTFAs, total TFAs, and n-3 PUFAs were significantly higher in men than in women, while total SFAs was higher in women compared to men. The odd-chain fatty acids, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), derived from dairy foods, were higher in women compared to men.

Table 2. Serum Phospholipid fatty acids in the population.

	Mean (95% CI) ^a N = 395	Mean (95% CI) ^a Men N = 129	Mean (95% CI) ^a Women N = 266	p Value ^b
Saturated fatty acids (SFAs)				
14:0 (myristic acid)	0.19 (0.18; 0.20)	0.17 (0.16; 0.19)	0.20 (0.18; 0.21)	0.04
15:0 (pentanoic acid)	0.14 (0.13; 0.143)	0.13 (0.11; 0.14)	0.14 (0.13; 0.15)	0.004
16:0 (palmitic acid)	23.16 (22.86; 23.46)	22.68 (22.01; 23.36)	23.40 (23.09; 23.70)	0.03
17:0 (heptadecanoic acid)	0.41 (0.40; 0.42)	0.39 (0.38; 0.41)	0.41 (0.40; 0.42)	0.02
18:0 (stearic acid)	15.13 (14.99; 15.26)	14.98 (14.77; 15.19)	15.20 (15.02; 15.37)	NS
Monounsaturated fatty acids (MUFAs)				
<i>cis</i> -MUFAs				
16:1n-7 (palmitoleic acid)	0.59 (0.57; 0.61)	0.56 (0.53; 0.59)	0.61 (0.58; 0.63)	0.03
18:1n-5	0.03 (0.029; 0.034)	0.037 (0.034; 0.04)	0.033 (0.031; 0.035)	NS
18:1n-7 (<i>cis</i> -vaccenic acid)	1.24 (1.22; 1.26)	1.23 (1.20; 1.27)	1.25 (1.22; 1.27)	NS
18:1n-9 (oleic acid)	9.12 (8.99; 9.25)	9.40 (9.17; 9.64)	8.99 (8.84; 9.15)	0.003
<i>trans</i> -MUFAs				
16:1n-7/9 (palmitelaidic acid)	0.22 (0.21; 0.23)	0.22 (0.21; 0.23)	0.22 (0.21; 0.23)	NS
18:1n-9/12 (elaidic acid)	0.14 (0.13; 0.15)	0.14 (0.13; 0.15)	0.14 (0.129; 0.145)	NS
18:1n-7 (vaccenic acid)	0.03 (0.02; 0.04)	0.07 (0.06; 0.08)	0.065 (0.061; 0.069)	NS
Polyunsaturated fatty acids (PUFAs)				
<i>cis</i> n-6 PUFAs				
18:2n-6 (linoleic acid)	24.42 (24.12; 24.72)	24.89 (24.32; 25.48)	24.19 (23.84; 24.54)	0.03
18:3n-6 (γ -linolenic acid)	0.16 (0.15; 0.17)	0.16 (0.14; 0.17)	0.16 (0.15; 0.17)	NS
20:3n-6 (di-homo- γ -linolenic acid)	4.12 (4.02; 4.22)	3.86 (3.69; 4.03)	4.25 (4.12; 4.37)	0.0003
20:4n-6 (arachidonic acid)	13.49 (13.25; 13.74)	13.39 (12.94; 13.87)	13.53 (13.25; 13.82)	NS
22:4n-6 (adrenic acid)	0.60 (0.59; 0.61)	0.61 (0.59; 0.63)	0.59 (0.58; 0.61)	NS
22:5n-6 (osbond acid)	0.47 (0.46; 0.49)	0.44 (0.42; 0.47)	0.49 (0.47; 0.51)	0.003
<i>Trans</i> -n-6 PUFAs				
Conjugated linoleic acid (CLA)	0.078 (0.07; 0.08)	0.077 (0.071; 0.083)	0.078 (0.074; 0.082)	NS
18:2ct, 18:2tc, 18:2tt (<i>trans</i> linoleic acid)	0.89 (0.80; 0.90)	0.099 (0.092; 0.11)	0.081 (0.077; 0.085)	<0.0001
<i>cis</i> -n-9 PUFA				
20:3n-9 (mead acid)	0.095 (0.092; 0.098)	0.090 (0.084; 0.097)	0.096 (0.092; 0.10)	0.04
<i>cis</i> -n-3 PUFA				
18:3n-3ccc (α -linolenic acid)	0.11 (0.11; 0.12)	0.12 (0.11; 0.13)	0.11 (0.10; 0.12)	NS
20:5n-3 (eicosapentaenoic acid, EPA)	0.30 (0.29; 0.32)	0.32 (0.29; 0.36)	0.30 (0.28; 0.31)	NS
22:5n-3 (docosapentaenoic acid, DPA)	0.71 (0.69; 0.73)	0.78 (0.74; 0.81)	0.68 (0.66; 0.70)	<0.0001
22:6n-3 (docosahexaenoic acid, DHA)	2.84 (2.77; 2.91)	2.89 (2.76; 3.03)	2.82 (2.73; 2.90)	NS

Table 2. Cont.

	Mean (95% CI) ^a N = 395	Mean (95% CI) ^a Men N = 129	Mean (95% CI) ^a Women N = 266	p Value ^b
<i>Trans</i> -n-3 PUFAs				
18:3n-3cct, ctt, ttt (<i>trans</i> α-linolenic acid)	0.01 (0.008; 0.012)	0.01 (0.014; 0.017)	0.01 (0.015; 0.017)	NS
Groupings				
Total SFAs	39.25 (39.02; 39.47)	38.65 (38.16; 39.14)	39.54 (39.32; 39.77)	<0.0001
Total <i>cis</i> -MUFAs	11.34 (11.19; 11.48)	11.58 (11.33; 11.84)	11.22 (11.05; 11.39)	0.02
Total <i>trans</i> ruminant fatty acids	0.14 (0.13; 0.15)	0.15 (0.14; 0.16)	0.14 (0.13; 0.145)	NS
Total <i>trans</i> industrial fatty acids	0.49 (0.48; 0.50)	0.50 (0.48; 0.52)	0.48 (0.47; 0.50)	0.007
Total <i>trans</i> fatty acids	0.64 (0.63; 0.66)	0.66 (0.63; 0.69)	0.63 (0.61; 0.65)	0.04
Total <i>cis</i> n-6 PUFAs	44.23 (44.00; 44.46)	44.34 (43.86; 44.83)	44.17 (43.92; 44.42)	NS
Total long-chain n-6 PUFAs	19.33 (19.07; 19.59)	18.93 (18.45; 19.43)	19.52 (19.22; 19.82)	0.04
Total <i>cis</i> n-3 PUFAs	4.10 (4.01; 4.19)	4.24 (4.07; 4.42)	4.03 (3.93; 4.13)	0.03
Total long-chain n-3 PUFAs	3.97 (3.89; 4.06)	4.11 (3.94; 4.29)	3.91 (3.81; 4.01)	0.04
Long-chain n-6/n-3 PUFAs	4.86 (4.74; 4.97)	4.61 (4.41; 4.82)	4.99 (4.85; 5.13)	0.002
Ratio n-6/n-3 PUFAs	10.78 (10.53; 11.04)	10.46 (10.00; 10.94)	10.94 (10.65; 11.25)	NS
Desaturation indexes				
Desaturation index ₁₆ (16:1n-7 <i>cis</i> /16:0)	0.026 (0.025; 0.026)	0.025 (0.024; 0.026)	0.026 (0.025; 0.027)	NS
Desaturation index ₁₈ (18:1n-9 <i>cis</i> /18:0)	0.60 (0.59; 0.61)	0.63 (0.61; 0.65)	0.59 (0.58; 0.60)	0.003

^a Fatty acids are expressed as a percentage of total fatty acids and represented as geometric means with 95% confidence intervals (CIs); ^b Independent-sample *t*-test.

3.3. Correlation between Serum Fatty Acids and Indicators of Obesity

Tables 3 and 4 show the Spearman coefficients of correlation between fatty acid families and indicators of obesity, BMI, and waist circumference.

BMI was significantly positively correlated with total SFAs in both men ($r = 0.40$, $p < 0.0001$, $q < 0.0001$) and women ($r = 0.33$, $p < 0.0001$, $q < 0.0001$) (p of heterogeneity = 0.035). In terms of individual SFA, no significant correlation was found between BMI or waist and the odd-chain fatty acids, pentadecanoic acid (15:0) and heptadecanoic acid (17:0). Similar trends were found with waist circumference (Table 4) and with percentage of body fat (data not shown).

Table 3. Partial Spearman ^a correlation between serum phospholipid fatty acids and BMI.

Fatty Acids (Percentage of Total Fatty Acids)	Men (N = 129)			Women (N = 266)			p of Heterogeneity
	r	p	q ^b	r	p	q ^b	
Saturated fatty acids (SFAs)							
Pentadecanoic acid (15:0)	0.17	0.06	0.26	-0.009	0.88	0.88	NS
Heptadecanoic acid (17:0)	-0.09	0.30	0.44	-0.11	0.07	0.12	NS
Palmitic acid (16:0)	0.19	0.03	0.22	0.04	0.53	0.69	NS
Stearic acid (18:0)	0.11	0.22	0.43	0.26	<0.0001	<0.001	NS
Total SFA ^c	0.40	<0.0001	<0.001	0.33	<0.0001	<0.0001	0.03
<i>cis</i> -Monounsaturated fatty acids (MUFAs)							
Palmitoleic acid (16:1n-7,9)	0.18	0.049	0.24	0.15	0.01	0.03	NS
Oleic acid (18:1n-9)	-0.09	0.34	0.46	-0.20	0.001	0.006	NS
Total <i>cis</i> -MUFA ^d	-0.12	0.19	0.43	-0.20	0.001	0.006	NS
n-6 polyunsaturated fatty acids (PUFAs)							
Linoleic acid (18:2n-6)	-0.07	0.44	0.55	-0.05	0.45	0.64	NS
γ-Linolenic acid (18:3n-6)	0.05	0.57	0.65	0.17	0.006	0.03	NS
Arachidonic acid (20:4n-6)	-0.10	0.26	0.43	0.03	0.66	0.75	NS
Total n-6 PUFAs ^e	-0.09	0.29	0.44	-0.05	0.38	0.57	NS
Total long-chain n-6 PUFAs ^f	-0.03	0.73	0.75	0.04	0.48	0.65	NS
<i>cis</i> -n-3 PUFAs							
α-linolenic acid (18:3n-3)	0.18	0.04	0.24	-0.12	0.06	0.11	0.02
Eicosapentaenoic acid (EPA, 20:5n-3)	0.09	0.31	0.44	0.14	0.02	0.05	NS
Docosahexaenoic acid (DHA, 22:6n-3)	-0.24	0.009	0.10	-0.03	0.61	0.75	NS
Total n-3 PUFAs ^g	-0.14	0.12	0.35	-0.03	0.63	0.75	NS
Total long-chain n-3 PUFAs ^h	-0.16	0.08	0.26	-0.02	0.74	0.79	NS
Industrial <i>trans</i> fatty acids (iTfAs)							
Palmitelaidic acid (16:1n-9)	0.11	0.23	0.43	-0.12	0.05	0.10	NS
Elaidic acid (18:1n-9/12)	-0.14	0.13	0.35	-0.14	0.02	0.05	NS
Linoleic acid (18:2tt, ct, tc)	-0.04	0.62	0.66	-0.15	0.01	0.03	NS
α-Linolenic acid (18:3n-3ctt, ttc)	0.22	0.01	0.10	0.07	0.24	0.40	NS
Total iTfAs	0.009	0.92	0.92	-0.17	0.007	0.03	NS
Ruminant <i>trans</i> fatty acids (rTfAs)							
Vaccenic acid (18:1n-7)	-0.16	0.07	0.26	-0.12	0.05	0.11	NS
Conjugated linoleic acids (CLAs, 9c-11t; 10t, 12c)	0.05	0.59	0.65	-0.06	0.32	0.51	NS
Total rTfAs	-0.05	0.54	0.65	-0.11	0.06	0.11	NS

Table 3. Cont.

	Men (N = 129)			Women (N = 266)			
Ratio							
n-6 PUFAs/n-3 PUFAs	0.10	0.26	0.43	0.018	0.77	0.79	NS
Long-chain n-6 PUFA/long-chain n-3 PUFAs	0.08	0.38	0.49	0.02	0.68	0.75	NS
Desaturation index ₁₆ (DI ₁₆ , 16:1/16:0)	0.12	0.20	0.43	0.13	0.03	0.07	NS
Desaturation index ₁₈ (DI ₁₈ , 18:1/18:0)	−0.11	0.22	0.43	−0.26	<0.001	<0.001	0.03

^a Models were adjusted for age, alcohol consumption, smoking, energy intake, education, physical activity, menopausal status in women, and batch of analysis. ^b Value for FDR (False Discovery Rate) correction. ^c Total SFA included 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; ^d Total *cis*-MUFA included 14:1, 15:1, 16:1n-7,9, 17:1, 18:1n-5, 7, 9, 20:1, 22:1, 24:1; ^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.

Table 4. Partial Spearman ^a correlations between serum phospholipid fatty acids and waist circumference.

Fatty acids (Percentage of Total Fatty Acids)	Men (n = 129)			Women (n = 266)			p of Heterogeneity
	r	p	q ^b	r	p	q ^b	
Saturated fatty acids (SFAs)							
Pentadecanoic acid (15:0)	0.15	0.09	0.24	−0.0007	0.99	0.99	NS
Heptadecanoic acid (17:0)	−0.13	0.14	0.28	−0.06	0.32	0.48	NS
Palmitic acid (16:0)	0.23	0.01	0.06	0.01	0.86	0.94	NS
Stearic acid (18:0)	0.03	0.77	0.77	0.26	<0.0001	<0.001	0.04
Total SFAs ^c	0.37	<0.0001	<0.001	0.27	<0.0001	<0.001	NS
<i>cis</i> -Monounsaturated fatty acids (MUFAs)							
Palmitoleic acid (16:1n-7,9)	0.20	0.03	0.12	0.20	0.001	0.006	NS
Oleic acid (18:1n-9)	−0.04	0.66	0.74	−0.10	0.11	0.25	NS
Total <i>cis</i> -MUFAs ^d	−0.076	0.41	0.61	−0.09	0.13	0.26	NS
n-6 polyunsaturated fatty acids (PUFAs)							
Linoleic acid (18:2n-6)	−0.06	0.54	0.70	−0.12	0.06	0.16	NS
γ-Linolenic acid (18:3n-6)	0.05	0.56	0.70	0.24	0.0001	0.001	NS
Arachidonic acid (20:4n-6)	−0.14	0.12	0.26	0.05	0.40	0.52	NS
Total n-6 PUFAs ^e	−0.11	0.25	0.42	−0.11	0.08	0.20	NS
Total long-chain n-6 PUFAs ^f	−0.07	0.47	0.67	0.07	0.23	0.41	NS
<i>cis</i> -n-3 PUFAs							
α-Linolenic acid (18:3n-3)	0.19	0.04	0.12	−0.07	0.25	0.41	0.027
Eicosapentaenoic acid (EPA, 20:5n-3)	0.03	0.72	0.75	0.21	<0.001	0.004	NS
Docosahexaenoic acid (DHA, 22:6n-3)	−0.25	0.006	0.06	0.003	0.96	0.99	NS
Total n-3 PUFAs ^g	−0.18	0.04	0.12	0.05	0.44	0.55	NS
Total long-chain n-3 PUFAs ^h	−0.20	0.03	0.12	0.06	0.36	0.51	NS
Industrial <i>trans</i> fatty acids (ITFAs)							
Palmitelaidic acid (16:1n-9)	0.10	0.28	0.44	−0.14	0.03	0.10	NS
Elaidic acid (18:1n-9/12)	−0.18	0.04	0.12	−0.10	0.12	0.26	NS
Linoleic acid (18:2tt, ct, tc)	−0.06	0.52	0.70	−0.13	0.04	0.12	NS

Table 4. Cont.

Fatty acids (Percentage of Total Fatty Acids)	Men (n = 129)			Women (n = 266)			p of Heterogeneity
	r	p	q ^b	r	p	q ^b	
α-Linolenic acid (18:3n-3ctt, ttc)	0.22	0.01	0.06	0.08	0.17	0.32	NS
Total rTFAs	−0.03	0.70	0.74	−0.14	0.02	0.07	NS
Ruminant <i>trans</i> fatty acids (rTFAs)							
Vaccenic acid (18:1n-7)	−0.24	0.008	0.06	−0.05	0.39	0.52	NS
Conjugated linoleic acids (CLA, 9c-11t; 10t,12c)	−0.04	0.67	0.74	−0.010	0.88	0.94	NS
Total rTFAs	−0.14	0.12	0.26	−0.04	0.52	0.62	NS
Ratio							
n-6 PUFAs/n-3 PUFAs	0.14	0.11	0.26	−0.07	0.28	0.44	NS
Long-chain n-6 PUFAs/long-chain n-3 PUFAs	0.12	0.19	0.36	−0.03	0.62	0.71	NS
Desaturation index ₁₆ (DI ₁₆ , 16:1/16:0)	0.11	0.22	0.39	0.19	0.02	0.06	NS
Desaturation index ₁₈ (DI ₁₈ , 18:1/18:0)	−0.03	0.71	0.75	−0.19	0.002	0.01	0.04

^a Models were adjusted for age, alcohol consumption, smoking, energy intake, education, physical activity, menopausal status in women, and batch of analysis. ^b Value for FDR correction.

^c Total SFA included 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0; ^d Total *cis*-MUFA included 14:1, 15:1, 16:1n-7,9, 17:1, 18:1n-5, 7, 9, 20:1, 22:1, 24:1; ^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; 18:2tt,ct,tc is a mixture of *trans*, *trans*, *cis*, *trans* and *trans*, *cis* isomers; 18:3n-3ctt, ttc is a mixture of *cis*, *trans*, *trans*, and *trans*, *trans*, *cis* isomers.

In women particularly, BMI was significantly positively correlated with MUFA palmitoleic acid ($r = 0.15$, $p = 0.01$, $q = 0.03$). A weak positive association was also reported with the DI_{16} ($r = 0.13$, $p = 0.03$, $q = 0.07$), but that did not withstand correction for multiple testing. Further adjustment for palmitic acid and palmitoleic acid did not change the correlation (data not shown). In contrast, a negative correlation was found in women between BMI and total iTFAs ($r = -0.17$, $p = 0.007$, $q = 0.03$). When we distinguished individual TFA isomers, we found differential correlations with BMI according to gender, with elaidic acid ($r = -0.14$, $p = 0.02$, $q = 0.05$), and *trans* linoleic acid ($r = -0.15$, $p = 0.01$, $q = 0.03$) showing significant inverse correlations in women, while *trans* isomers of α -linolenic acid showed a positive trend in men ($r = 0.22$, $p = 0.01$, $q = 0.10$). Similar trends were found with waist circumference (Table 4) and with percentage of body fat (data not shown).

No significant correlation was found between n-6 PUFAs, n-3 PUFAs, or the ratio n-6/n-3 PUFAs and BMI, waist circumference, and percentage of body fat (data not shown). When considering the ratio, further adjustment for n-6 and n-3 PUFAs did not change the correlation (data not shown). Divergent correlations according to gender were found between individual n-6 and n-3 PUFAs and BMI. In men, n-3 α -linolenic acid, the essential fatty acid of the n-3 family, tended to be positively correlated with obesity ($r = 0.18$, $p = 0.04$, $q = 0.24$), while a negative trend was found for long-chain n-3 docosahexaenoic acid (DHA) ($r = -0.24$, $p = 0.009$, $q = 0.10$). In women, n-6 γ -linoleic acid ($r = 0.17$, $p = 0.006$, $q = 0.03$) and n-3 eicosapentanoic acid (EPA) ($r = 0.14$, $p = 0.02$, $q = 0.05$) were positively correlated with BMI.

Similar correlations with BMI, waist circumference, and percentage of body fat were found when fatty acids were expressed in amounts (data not shown).

All individual fatty acids which were significantly correlated with indicators of obesity showed a significant linear relationship with BMI and waist. A non-statistically significant linear relationship was found between BMI or waist and all other individual fatty acids, such as pentadecanoic acid (15:0), heptadecanoic acid (17:0), and most of the n-6 and n-3 PUFAs (data not shown).

4. Discussion

This is the first population-based study reporting serum phospholipid fatty acid profiles in a Lebanese population and their correlations with indicators of adiposity. We found that total levels of SFAs were positively correlated with BMI in both men and women. Palmitoleic acid and DI_{16} , as biomarkers of endogenous lipogenesis, were positively correlated with BMI, particularly in women. Divergent correlations were reported between individual *trans* fatty acids, n-6 and n-3 PUFAs, and BMI. Similar trends were found in relation to waist circumference and percentage of body fat. These findings suggest that different subtypes of fatty acids may differentially impact obesity. Further, we identified a specific fatty acid profile in this Lebanese population compared to other populations.

The measurement of serum phospholipid fatty acids is a complementary tool to estimate dietary fatty acid intake through dietary assessment methods. Serum or plasma phospholipid fatty acids represent specific biomarkers of past dietary intakes (weeks to months) of fatty acids that cannot be endogenously synthesized, such as PUFAs and iTFAs [19,21,22]. In contrast, weak associations were found between dietary intakes of SFAs and MUFAs and their respective levels in plasma phospholipid, likely because of endogenous synthesis of these fatty acids [19,22]. A significant positive association was found between plasma MUFAs or DI_{16} and dietary intakes of SFA, suggesting that blood phospholipid MUFAs are biomarkers of dietary SFA and endogenous lipogenesis [19,22]. Thus, SFA and MUFA levels in blood phospholipid fraction among free-living individuals are likely to be markers of both dietary intake and de novo lipogenesis [19,23].

It is challenging to determine whether the distribution of various fatty acids in a given population is low or high due to a lack of appropriate reference values. As an alternative, we compared serum phospholipid fatty acid profiles in Lebanese adults to those reported in participants from the Mediterranean regions (Athens, Spain, and Ragusa/Naples) in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, based on data measured in our laboratory using the same

methodology [22]. Compared to Mediterranean European adults, the fatty acid profile of Lebanese adults is markedly different, particularly regarding PUFA levels. The most prominent difference in Lebanon is a higher level of n-6 PUFA (44.23% in Lebanon versus 38.95% in Mediterranean regions in the EPIC study), presumably originating from vegetable oils, and low levels of n-3 PUFA derived from fish (4.10% vs. 7.57%). Accordingly, the ratio of n-6/n-3 PUFA in the Lebanese population is much higher than in Mediterranean regions of EPIC (10.78 vs. 5.15) [22]. Levels of MUFA (11.34% vs. 13.71%) are lower in Lebanese adults than in Mediterranean European adults. In contrast, total levels of SFA in Lebanon individuals were found comparable to those reported in Mediterranean regions of EPIC (39.25% of total fatty acids in Lebanon versus vs. 39.76% in Mediterranean regions of EPIC) as well as the levels of *trans* elaidic acid (0.14% vs. 0.15%). Even if the two study populations differ on different characteristics, for example mean age at recruitment (47.3 years in the Lebanese study vs. 53.9 years in EPIC), mean BMI at recruitment (29.6 in the Lebanese study vs. 25.5 in the EPIC study) or date at blood collection (2014 in the Lebanese cohort vs. 1992–1998 for the EPIC study), we previously showed that geographic region appeared to be the strongest determinant factor explaining variability in blood levels of fatty acids [22].

We found that total SFAs were significantly positively correlated with BMI and waist circumference, which was somewhat stronger in men than in women. Similar trends between total SFAs and BMI have been reported in a cross-sectional analysis among Mexican women [24]. In agreement with our findings, a review of epidemiological studies and clinical trials described that SFA consumption led to increased body adiposity [25]. Similarly, SFA intake has been linked to obesity and specifically to abdominal fat accumulation among women enrolled in the Nurses' Health study [26] and among U.S. men in another prospective study [25]. We found that the positive correlations between total SFAs and BMI or waist circumference are likely to be driven by palmitic acid in men and stearic acid in women. In contrast, among odd-chain saturated fatty acids originating from dairy foods, heptadecanoic acid showed a non-significant inverse association with BMI and waist circumference in women. These data suggest that individual SFAs may have differential effects on adiposity depending on their dietary sources and endogenous synthesis. Furthermore, we found that palmitoleic acid and DI_{16} , as biomarkers of endogenous lipogenesis, were positively correlated with BMI, particularly in women. Our data further suggest that an increased endogenous synthesis of palmitoleic acid may increase adiposity. In agreement with our findings, some epidemiological studies have consistently reported a positive association between adipose tissue or circulating palmitoleic acid, DI_{16} and obesity [11–15]. Furthermore, an epidemiological study among Japanese employees indicated that high levels of serum palmitoleic acid levels led to increased concentrations of C-peptide, insulin resistance, and inflammation [27], known factors involved in obesity. Our data suggest that increased endogenous synthesis of palmitoleic acid may increase adiposity. Further studies are needed to explore the causality of the association between increased synthesis of palmitoleic acid and obesity, and whether this effect might be mediated by insulin resistance and inflammation.

Inconsistent trends were found in this study between levels of total iTFAs, individual iTFA isomers, and BMI or waist circumference among women, albeit these correlations were weak. When we distinguished individual TFA isomers, we found differential correlations with BMI according to gender, with elaidic acid and *trans* linoleic acid showing significant inverse correlations in women, while *trans* isomers of α -linolenic acid showing a positive trend in men. Similar to our finding, a cross sectional analysis among Costa Rican adults reported divergent associations between TFA isomers and adiposity [28]. In particular, negative associations between *trans* isomers of 18:1 (as the sum of 18:1n-7, 18:1n-9, and 18:1n-11) measured in adipose tissue and all measures of adiposity (visceral and subcutaneous adiposity) were reported [28]. This inverse association was explained by the relatively low consumption of *trans* isomers of 18:1 in Costa Rica [28]. Also, no clear association was observed between plasma phospholipid levels of total *trans* fatty acids (as the sum of 16:1, 18:1, and 18:2) and baseline BMI or BMI changes (during 10 years of follow-up) in a cross-sectional and longitudinal study with available repeated measurements within the American Multi-Ethnic Study of Atherosclerosis

(MESA) cohort [29]. In contrast, high baseline blood levels of iTFA elaidic acid have been associated with an increased risk of weight gain during a 5-year follow-up in the European EPIC cohort [16]. In agreement with this finding, a significant positive association between levels of total *trans* 18:1 measured in erythrocyte membranes and weight gain was reported in American women during a 10.4-year follow-up [30]. Data from experimental models suggested that iTFA may induce obesity. A long-term intervention study on primates reported an increase of body weight in animals receiving an iTFA diet compared to those receiving *cis*-fatty acids [31,32]. Another study showed that a diet high in *trans* fat induces insulin resistance pathway and obesity [33]. The association between iTFA and obesity still remains unclear and needs further investigation in prospective settings.

N-6 and n-3 PUFAs may have divergent effects on the development of obesity through their differential effect on inflammation [34]. In our study population, divergent correlations according to gender were reported between individual n-6 and n-3 PUFAs and obesity. In men, n-3 α -linolenic acid, the essential fatty acid of the n-3 family, tended to be positively correlated with obesity, while a negative trend was found for long-chain n-3 docosahexaenoic acid (DHA). In women, n-6 γ -linoleic acid and n-3 eicosapentanoic acid (EPA) were positively correlated with obesity. A similar positive trend between EPA and BMI was reported in a cross-sectional study among Mexican women [17]. Similarly, a positive association was found between levels of EPA in blood cholesterol esters and abdominal obesity in Swedish women but not in men [15]. Although the ratio n-6/n-3 PUFAs in our study is high (10.78), no significant correlation was found with indicators of obesity. In contrast, n-6/n-3 PUFAs was associated with an increased risk of weight gain in the Women Health Initiative (WHI) study [30], despite the fact that this ratio was much lower in this population (4.68) compared to the ratio reported in the present study. This discrepancy between studies might be the consequence of the design, prospective versus cross-sectional, of the levels of PUFAs reported in each population, and of the sample size of each study. Further studies with a prospective design are needed to investigate the association between n-6 and n-3 PUFAs, as well as the ratio n-6/n-3 PUFAs, and obesity.

This study has characterized the serum phospholipid fatty acid profile in a Lebanese population and highlighted important differences with a European population living in Mediterranean regions. However, the findings of this study are limited by the cross-sectional nature of the analysis and these data need to be replicated in a prospective setting.

5. Conclusions

In conclusion, this study suggests that high blood levels of some SFAs and MUFA palmitoleic acid, likely to derive from dietary intake of SFA and increased endogenous lipogenesis, is correlated with increased adiposity in the Lebanese population. The causality of these associations remains to be investigated. Reducing SFA intakes could potentially offer a public health strategy for reducing BMI. In addition to being the first of its kind in the Middle East, this report provides a timely framework to examine biomarkers and health effects in a region currently undergoing a nutritional transition.

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Chapter 7. General conclusion

7.1. Main findings and overall discussion

Since the beginning of the 20th century, when the role of food and nutrition in health and disease became evident, dietary recommendations have been part of public health messages and have helped in the improvement of the quality of diet. Against the growing cancer burden worldwide, this improvement may be of utmost importance in prevention strategies. Dietary foods and nutritional patterns, including FA, have been shown to be associated to several cancer risks, including OC and EC, the incidence of which are rising worldwide. This work aimed to identify dietary risk factors for these two cancers with a particular attention on FA, and help in promoting nutritional recommendations aiming to reduce the cancer burden.

Countries face today substantial challenges in implementing prevention strategies worldwide, which threatens economies and impoverishes individuals and families, particularly in LMICs. Our characterization of FA biomarkers in relation to indicators of obesity in a Lebanese population represents the first study of this kind in the Middle East and North Africa region, providing a baseline that can be further exploited in future studies aiming to address cancer prevention strategies, particularly that obesity is a well-characterized cancer risk factor (including for OC and EC).

Our findings in the European EPIC study suggested that higher dietary intakes of iTFA, may be associated with greater risk of EOC. Similar associations were found with circulating fatty acids as biomarkers of their dietary intakes. Furthermore, the risk increased at dietary intakes of iTFA below dietary limits of 1% of total energy intake recommended by WHO (0.87% of total energy intake).

Furthermore, dietary intakes of *cis* n-6 linoleic acid and n-3 α -linolenic acid were also positively associated with EOC risk in this study. According to our investigations on the dietary sources of these two FA, these positive associations were mainly driven by deep frying fat.

This positive association might be due to co-exposure to other compounds occurring in food processing and/or cooking due to chemical alterations occurring during deep frying fat such as aldehydes, oxidized lipids, heterocyclic compounds, TFA, polymers, sterol derivatives, acrylamide, and acrolein (128).

Perspectives

While our findings highlighted a possible association between iTFA, the use of deep-frying fat and EOC risk, future studies are needed to further replication and clarification. As a matter of fact, this study was limited by the single collection of questionnaires and blood samples at baseline. In addition, food composition data as well as dietary intake data may be prone to measurement error. However, relative validation analysis comparing FA intakes with biomarkers confirmed high quality of the dietary intake estimates of the individual FA (correlation between dietary and plasma phospholipid iTFA=0.45 in our study compared to 0.25 in in the Women's Lifestyle Validation Study (169)).

To further complete and confirm results suggested during this work, these analyses might be replicated in other cohorts like the recent French cohort Nutrinet-Santé, with detailed information on ultra-processed foods and like cohorts in industrialised countries like the US where the intake of iTFA is high. Similar studies should also be set up in some LMIC where adoption of unhealthy diets is increasing, as well as occurrence of cancers. Furthermore, Mendelian Randomisation (MR) analyses also performed in our section are potential tools to assess causal associations between FA and EOC risk.

Our findings in the EC study suggested an inverse association between n-6 γ -linolenic acid and EC risk that was mainly driven by its vegetable sources. In addition, an inverse association was also found between the vegetable sources of n-3 α -linolenic acid and EC risk.

These data suggest that the dietary source of FA (animal versus vegetal) is determinant when investigating the association between FA and cancer risk.

Regarding iTFA, there is a lack of associations between iTFA and EC risk in the literature (111, 112), as well as in our study. Further studies in populations with highest consumption of iTFA, might reveal a possible association between *trans*-fat and EC risk.

To our knowledge, no previous studies have investigated the associations between animal and plant sources of FA and EC risk. Only one recent study in the NHS and HPFS has reported that higher intake of MUFA from plant sources was associated with lower risk of total mortality (145). Regarding n-6 γ -linolenic acid, no studies have examined its association with EC risk. A recent study has reported that inflammatory breast cancer was associated with decreased levels of n-6 γ -linolenic acid in breast adipose tissue (145), this n-6 PUFA being known to be precursor of anti-inflammatory prostaglandins 1-series. Thus, this inverse association between γ -linolenic acid and EC risk might be mediated by anti-inflammatory processes.

A pooled analysis of three Italian case-control studies has reported beneficial role of the Mediterranean diet on EC risk, suggesting a favorable effect of a combination of foods rich in antioxidants, fibres, phytochemicals, and unsaturated FA (170). In another meta-analysis of observational and randomized trial studies, Mediterranean diet was associated with a lower risk of overall mortality, CVD and over-all cancer incidence; however no evidence was reported for EC (170). Knowing that EC is an obesity-related cancer (171), and that Mediterranean diet is the most appropriate regime to prevent obesity and other metabolic and chronic diseases (172), therefore such a diet rich in plant sources might reduce EC risk. However, further studies are needed to confirm this potential protective role.

Perspectives

While our findings emphasised a possible association between some FA from plant sources and EC risk, further replications and clarifications are needed, and this question might be addressed in Mediterranean populations.

To potentially complete this work, plasma PL-FA might be analysed in association with EC in a sub-EPIC. Even with the improvement of the EPIC dietary database, the biomarkers methodology, a more objective measurement, will still allow us to study the endogenous synthesis of FA in relation to this cancer, as reported in association with breast cancer in numerous epidemiological studies. Mediation analysis could be also performed to reveal a possible mediator role of inflammatory factors (CRP and IL6) in the association between FA and EC, an inflammatory disease. Further replications are also needed in other populations to confirm the association found in this EPIC study. To assess causality, MR analyses could be performed.

7.2. Final conclusion & Public health messages

In line with the WHO REPLACE initiative (129), eliminating iTFA intake through a regulation on industrial processes and on the use of deep-frying fat could potentially offer an effective public health action for reducing EOC risk, in addition to other chronic diseases (CVD and metabolic diseases). The risk of EC may be as well reduced by following the European Code Against Cancer (ECAC) developed and coordinated by IARC and WCRF recommendations on adherence to a diet rich in whole grains, pulses, vegetables and fruits and low in high-calorie foods (foods high in sugar or fat), sugary drinks, red and processed meat and salt.

My work in the field of FA and gynecological cancers may provide evidence of a possible association between some FA and these cancer risks and help to implement recommendations particularly on eliminating iTFA from diet and enriching vegetable intakes.

This field of nutrition, epidemiology and cancer provides evidence-based approaches capable of informing public health action and offers, at a personal level, a diversity of expertise and interdisciplinary skills. This expertise may allow me to pursue my research in this field.

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