UNIVERSITAT DE BARCELONA

Influence of menstrual factors, reproductive history and exogeneous hormone use on women's health: age at natural menopause, and cancer of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma

Leila Luján Barroso

Influence of menstrual factors, reproductive history and exogenous hormone use on women's health: age at natural menopause, and cancers of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma.

Doctoral Thesis presented by

LEILA LUJÁN BARROSO

To obtain the PhD degree

under the direction of

Dr. Raúl Zamora Ros

and

Dr. Esteve Fernández Muñoz

Thesis registered at the Doctoral Program of Medicine

University of Barcelona

2020

A totes les persones que estimo
The rest is still unwritten…

Cover design: Adrian Alescio

ICO
Institut Català d'Oncologia

UNIVERSITAT DE BARCELONA

Los doctores **Raúl Zamora Ros** y **Esteve Fernández Muñoz** como directores de la tesis de la doctoranda **Leila Luján Barroso** autorizamos el depósito y la presentación cuando la comisión de doctorado lo aprueba de la tesis doctoral titulada: **"Influence of menstrual factors, reproductive history and exogenous hormone use on women's health: age at natural menopause, and cancers of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma."**

Y para que conste a los efectos oportunos firmamos la presente

Barcelona, 10 de desembre de 2019

Doctoranda

Fdo: Leila Luján Barroso

Directors de la tesi

Fdo: Esteve Fernandez Muñoz

Fdo: Raúl Zamora Ros

ACKNOWLEDGMENTS

Aquesta és la secció que més em costava d'escriure… no per què no tingui a qui agrair, sinó perquè ha estat un camí llarg amb pedres i canvis. Finalment, vaig trobar el suport necessari per dur a termini la tesis.

Primer de tot, vull agrair-li a l'Eric Duell els anys que vam compartir com a director-estudiant, vaig aprendre moltes coses tant a nivell professional com a nivell personal. Gràcies al seu primer recolzament avui estic aquí.

En segon lloc, vull agrair a l'Antonio Agudo, i als meus directors Esteve Fernández y Raúl Zamora la confiança dipositada en mi en el moments més difícils del desenvolupament de la tesi. Si no hagués estat per ells segurament no l'hagués acabada.

Per continuar una menció especial alguns dels meus companys d'aquí l'ICO, la Mireia, l'Olga, la Laura C, la Laura E, la Marisa, el Jordi, el Ion i el Jesús. Són molts records que ens uneixen tant a nivell laboral com personal, hores d'esmorzar, de dinar etc. Tots vosaltres heu viscut amb mi molts dels canvis que he tingut aquest últims anys i heu estat la ma que sempre m'ha ajudat a aixecar-me, moltes gràcies. No m'oblido de la Bea, la Noemie, la Yolanda y la Delphin (gràcies per l'ajuda en el tema de linfomes). En general vull agrair a tots el companys del PREC tots aquest anys.

Vull agrair també a l'Elena, la Lorena, la Marta, la Montse i l'Alba i per extensió a les compis de bollywood. Tots els dimecres ens reunim ballem i m'ajudeu a distreure'm a oxigenar-me i expressar-me d'una altra manera. Gràcies pels moments pots-ball també i sento no haver pogut anar a Mallorca, sou unes campiones.

Masques, que dir-vos a vosaltres, sou el costat bo d'un dels grans canvis. Amb vosaltres em vaig iniciar al corre, i mira ja he fet fins una marató. Sou molt importants a la meva vida i aquest any vinent tindrem una nova incorporació. Felicitats Ester.

Raquel, tu junto a Elena, eres de las persones que más tiempo hace que me conoce. Hemos crecido juntas, compartido muy buenos momentos y siempre pasase lo que pasase hemos conseguido sortear las dificultades. Gracias por todos estos años, y los que nos quedan, de amistad. Elena, tú estás incluida en todos los bloques de

agradecimientos, baile, masques, colegio, universidad, creo que lo único que no hemos hecho juntas es trabajar. Nuevamente gracias por todo.

Sigo con la familia, a mis yayas y a ti Poto-Poto (espero que estés orgulloso de mi allí donde te encuentres, te echo mucho de menos), a todos mis tíos y tías, mis primas y primos, a todos gracias os quiero mucho, tengo una familia fabulosa y muy divertida, no la cambiaría por nada. Mis padres hasta hace unos días que no se han enterado que estaba realizando la tesis. Como siempre dicen voy a la chita callando; pero, aunque no lo supieran notaba su apoyo su cariño. Cristina, gracias por ser la mejor hermana, dama de honor y amiga que pueda pedir (aunque a veces nos saquemos de quicio). Gracias por tu ayuda con el inglés.

Por último, Adri hace unos meses ya te dije delante de todos nuestros amigos lo importante que eres para mí. Estos últimos meses de tesis has estado apoyándome en todo, siempre desde el amor y comprensión. Eres mi lugar favorito. Te quiero.

Leila.

Barcelona, 10 de desembre de 2019

PRESENTACIÓN DE LA TESIS

Esta tesis doctoral es el fruto del trabajo realizado durante los últimos cuatro años en la Unidad de Nutrición y Cáncer del Instituto Catalán de Oncología.

La tesis doctoral se presenta como una compilación de tres artículos publicados en revistas internacionales y de alto impacto y un artículo que se encuentra bajo revisión. En ellos se estudia el efecto de los factores menstruales y reproductores y la utilización de hormonas exógenas en la salud de la mujer. En concreto, en cómo afecta a la edad de la menopausia y en el riesgo de los cánceres de páncreas, de linfoma no Hodgkin y en el carcinoma urotelial. Los resultados presentados en esta tesis han sido realizados en el marco del Estudio Prospectivo Europeo sobre Cáncer y Nutrición y en el consorcio caso-control de cáncer de páncreas.

La tesis está escrita en inglés y sigue la estructura básica de introducción general, justificación, hipótesis y objetivos, metodología, resultados, discusión, conclusiones generales y referencias bibliográficas. Los anexos contienen las tablas suplementarias de los artículos. También incluyen los documentos aprobados del comité de ética del Hospital Universitario de Bellvitge y el *curriculum vitae* de la doctoranda.

LIST OF ABBREVIATIONS

FSH: Follicle-stimulating hormone

LH: Luteinizing hormone

InterLACE: International Collaboration for a Life Course Approach to Reproductive Health and Chronic Disease Events

OC: oral contraceptive

BMI: Body Mass Index

MHT: menopausal hormone therapy

WHI: Women's Health Initiative

IARC: International Agency of Research of Cancer

ER: estrogen receptor

HR: Hazard Ratio

CI: Confidence Interval

PanC4: Pancreatic Cancer Case-Control Consortium

EPIC: European Prospective Investigation into Cancer and Nutrition

UCSF: University of California, San Francisco

SEARCH: Surveillance of Environmental Aspects Related to Cancer in Humans

MDACC: MD Anderson Cancer Center

MSKCC: Memorial Sloan Kettering Cancer Center

OR: Odd ratio

arMED: adapted relative Mediterranean diet

IF: impact factor

EPIC-PANACEA: European Prospective Investigation into Cancer-Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating out of home And Obesity

LFHC: low-fat, high-carbohydrate dietary intervention study

PCOS: polycystic ovary syndrome

DOM-3: Doorlopend Onderzoek Morbiditeit/Mortaliteit cohort

InterLymph: International Lymphoma Epidemiology Consortium

IWHS: Iowa Women's Health Study

CTS: California Teachers Study

ABSTRACT IN ENGLISH

Background: Menopause is an inflexion point in women's health. Menopause is related to increased risk in osteoporosis and cardiovascular diseases. However, the risk of hormone-related cancers decreases after menopause. Tobacco smoking is the most established risk factor of earlier age at natural menopause. Educational level, physical activity, and oral contraceptive use (OC) use are potential modifiers of age at natural menopause. It has been shown that age at natural menopause varies across countries, but only few studies have included Spanish participants.

Incidence for cancers of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma is higher in men than in women. One possible explanation is due to the exposure to sex hormones, including estrogens, which may decrease female's incidence. Nevertheless, the influence of menstrual factors, reproductive factors and exogenous hormones use on the risk of pancreatic cancer and non-Hodgkin lymphoma is still unclear. Whereas, some reproductive factors, such as parity, independent of the number of births, and menopausal hormone therapy (MHT) have been already associated with lower urothelial carcinoma risk in few studies. Furthermore, an increased risk of urothelial carcinoma was observed in women with an earlier age at menopause.

Objective: to assess the role of menstrual factors, reproductive history, and exogenous hormones use on women's health, especially their influence on age at natural menopause, and cancers of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma.

Methods: we used two different studies and populations: the European Prospective Investigation into Cancer and Nutrition (EPIC) and the Pancreatic Cancer Case-Control Consortium (PanC4).

Results: to evaluate the role of lifestyle factors, diet, and menstrual and reproductive history, and OC use in age at natural menopause a total of 12,562 premenopausal women at recruitment were followed-up a median of 3 years. During the follow-up, 1,166 women became postmenopausal with a median age at natural menopause of 51 years. We observed that current smokers at baseline had a 29% higher risk of an earlier onset of menopause. Furthermore, we observed an inverse associations with age at

natural menopause in women with irregular menses during the first 10 years after the menarche (HR: 0.71; 95%CI: 0.56- 0.91), women with more than 1 full-term pregnancies ($HR_{\geq 4 vs 0}$: 0.74; 95%CI: 0.56- 0.94), and women who started the use of OC between the ages of 25 and 30 years compared to those who started after the age of 31 years (HR: 0.72; 95%CI: 0.58- 0.89).

The second sub-objective was to evaluate the role of menstrual factors, reproductive history, and exogenous hormones use in the risk of pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma. We observed an inverse association between pancreatic cancer and hysterectomized women who took MHT (OR: 0.64; 95%CI: 0.48- 0.84). Contrary, women with hysterectomy plus oophorectomy (especially bilateral oophorectomy) were positively associated with the risk of non-Hodgkin lymphoma (HR: 1.26; 95%CI: 1.01- 1.56). Finally, results on urothelial carcinoma showed a positive association between urothelial carcinoma and MHT-users compared to never-users (HR: 1.27; 95%CI: 1.03-1.57) and an inverse trend between the number of full-term pregnancies and the risk of urothelial carcinoma was shown ($HR_{\geq 5 vs 1}$: 0.48; 95%CI; 0.25- 0.90; *P* for trend in parous women only 0.010).

Conclusion: In conclusion, our results confirm that women who smoked had an earlier age at natural menopause; while use of OC, higher number of pregnancies, and irregularity of menses the first 10 years after menarche were associated with a prolonged reproductive lifespan. Menstrual and reproductive factors, and exogenous hormone use are unlike to play a role in the cancer risk of the pancreas and non-Hodgkin lymphoma. Lastly, we found that increasing number of full-term pregnancies might reduce urothelial carcinoma risk and limited evidence of the role of MHT-use in the risk of urothelial carcinoma.

RESUMEN EN CASTELLANO

Antecedentes: La menopausia es un punto de inflexión en la salud de la mujer y está relacionada con un aumento del riesgo de osteoporosis y enfermedades cardiovasculares. Sin embargo, el riesgo de los cánceres relacionados con los niveles hormonales desciende tras la menopausia. El factor de riesgo más conocido que adelanta la edad de la menopausia es el tabaco. El nivel educacional, la actividad física y el uso de anticonceptivos orales son potenciales modificadores de la edad de la menopausia. Ha sido visto que la edad de la menopausia varía según el país, pero pocos estudios han incluido participantes españolas.

La incidencia de los cánceres de páncreas, linfoma no Hodgkin y el carcinoma urotelial es mayor en hombres que en mujeres. Una posible explicación es que la exposición a las hormonas sexuales, incluyendo los estrógenos, pueden reducir la incidencia en la mujer. Sin embargo, la influencia de los factores menstruales y reproductivos y la utilización de hormonas exógenas en los riesgos de los cánceres de páncreas y linfoma de no Hodgkin aun no está clara. Sin embargo, algunos factores reproductores, como por ejemplo la paridad, independientemente del número de hijos, y el uso de terapia hormonal sustitutoria han sido relacionados con un riesgo menor de carcinoma urotelial en algunos estudios. Además, se observó un mayor riesgo de carcinoma urotelial en mujeres con una edad más temprana en la menopausia.

Objetivo: Evaluar el rol de los factores menstruales, la historia reproductiva y el uso de hormonas exógenas en la salud de la mujer, especialmente su influencia en la edad de la menopausia natural y en los cánceres de páncreas, linfoma no Hodgkin y carcinoma urotelial.

Métodos: Utilizamos dos diferentes estudios y poblaciones: Estudio Prospectivo Europeo sobre Cáncer y Nutrición y en el consorcio caso-control de cáncer de páncreas.

Resultados: Para evaluar el rol de los factores de estilo de vida, la dieta y la historia reproductiva y la utilización de anticonceptivos orales, un total de 12,562 mujeres pre menopaúsicas al reclutamiento fueron seguidas una mediana de 3 años. Al finalizar el seguimiento 1,166 mujeres habían alcanzado la menopausia de manera natural con una mediana de edad de 51 años. Observamos que las fumadoras actuales al reclutamiento tenían un riesgo un 29% mayor de un inicio temprano de la menopausia. Además,

observamos una asociación inversa en la edad de la menopausia natural en las mujeres con menstruaciones irregulares durante los primeros 10 años después de la menarquía (HR: 0.71; 95%IC: 0.56- 0.91), en las mujeres con más de 1 embarazo a término ($HR_{\geq 4 vs 0}$: 0.74; 95%CI: 0.56- 0.94) y en las mujeres que empezaron a utilizar los anticonceptivos orales entre las edades de 25 y 30 años comparadas con aquellas que empezaron después de los 31 años (HR: 0.72; 95%CI: 0.58- 0.89).

El segundo sub-objetivo fue evaluar el rol de los factores menstruales y reproductivos y el uso de hormonas exógenas en relación al riesgo del cáncer de páncreas, linfoma de no Hodgkin y el carcinoma urotelial. Observamos una asociación inversa entre el cáncer de páncreas y las mujeres histerectomizadas que tomaron terapia hormonal sustitutoria (OR: 0.64; 95%CI: 0.48- 0.84). Por el contrario, las mujeres con histerectomía y ovariotomía (especialmente la bilateral) fueron asociadas de manera positiva con el riesgo de linfoma de no Hodgkin (HR: 1.26; 95%CI: 1.01- 1.56). Finalmente, los resultados en carcinoma urotelial mostraron una asociación positiva entre el carcinoma urotelial y el uso de terapia hormonal sustitutoria comparado con las que nunca la usaron (HR: 1.27; 95%CI: 1.03-1.57) y observamos una tendencia inversa entre el número total de embarazos a término y el riego de carcinoma urotelial ($HR_{\geq 5 vs 1}$: 0.48; 95%CI; 0.25- 0.90; *P* de tendencia en mujeres que tuvieron hijos 0.010).

Conclusión: En conclusión, nuestros resultados confirmaron que las mujeres que fumaban tenían una edad más temprana en la menopausia natural; mientras que la utilización de anticonceptivos orales, mayor número de embarazos y tener menstruaciones irregulares los primeros 10 años después de la menarquía estuvo asociado con una vida reproductiva prolongada. Los factores menstruales y reproductivos y el uso de hormonas exógenas no desempeñan un papel importante en el riesgo del cáncer de páncreas y linfoma de no Hodgkin. Finalmente, encontramos que un número creciente de embarazos a término podría reducir el riesgo de carcinoma urotelial y una evidencia limitada del rol del uso terapia hormonal sustitutoria en el riego de carcinoma urotelial.

TABLE OF CONTENTS

List of Figures

List of Tables

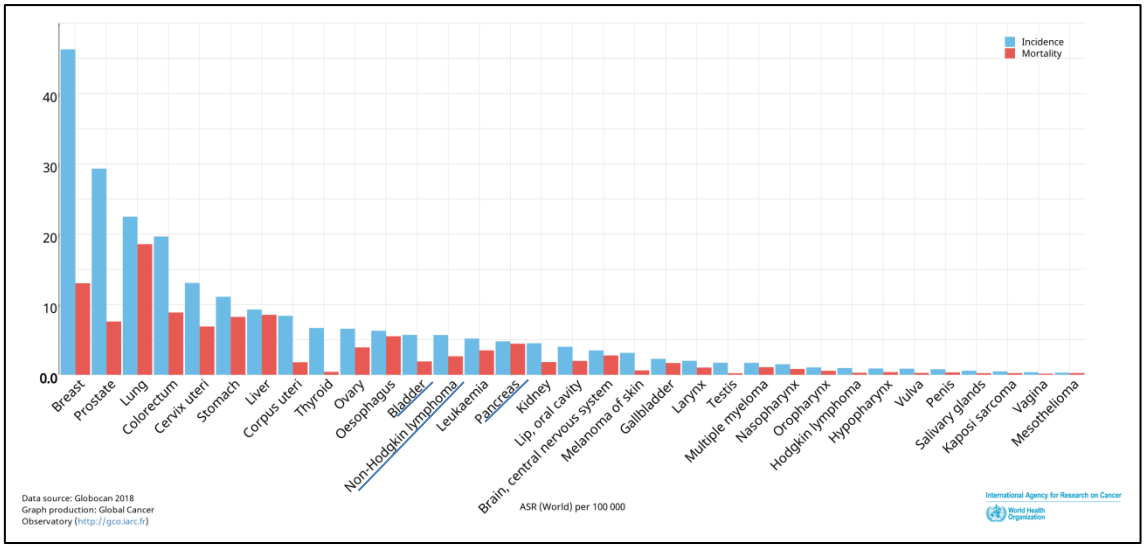
1. INTRODUCTION

1.1. Pancreatic cancer

1.1.1. Epidemiology

Pancreatic cancer was the 15th most common cancer and the 9th cause of cancer mortality worldwide (Figure 1) in 2018 (1). Age-adjusted incident rates of pancreatic cancer were generally higher at high-income countries; being the highest in Europe, followed by North America and Australia/New Zealand. While in Europe the incident rate was 7.7 per 100,000 inhabitants, in low-income countries it was 2.2 per 100,000 inhabitants. Furthermore, pancreatic cancer incidence was slightly higher in men than in women, in men age-adjusted incident rate worldwide was 5.5 and 4 per 100,000 inhabitants in men and women, respectively (1).

Figure 1: Estimated age-standardized incidence and mortality rates (per 100,000 inhabitants) worldwide in both sexes combined in 2018



Adapted form GLOBOCAN-IARC 2018 (1)

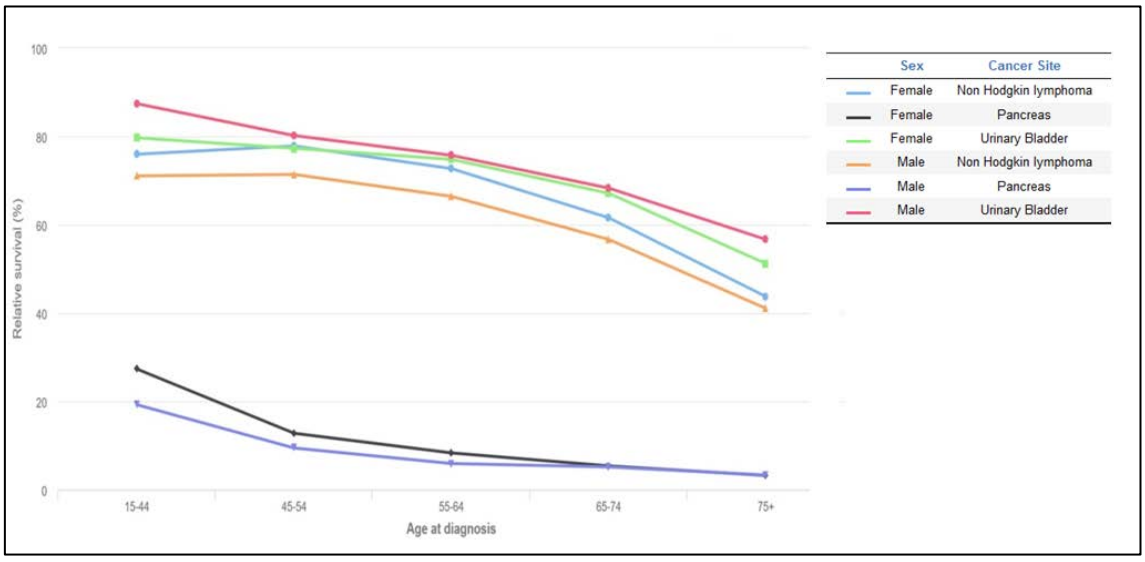
The most common pancreatic cancer histological subtype is ductal adenocarcinoma accounting for 95% of cases, the remaining 5% of exocrine cancers include

adenosquamous carcinomas, squamous cell carcinomas, signet ring cell carcinomas, undifferentiated carcinomas, and undifferentiated carcinomas with giant cells.

Ductal adenocarcinoma starts when the exocrine cells in the pancreas grow out of control. Pancreatic cancer is frequently diagnosed between the ages of 65 and 74, being the mean age at diagnosis 69 years for men and 72 years for women.

Early symptoms of pancreatic cancer could indicate different illnesses of the abdomen or gastrointestinal tract. Some of these symptoms consist of abdominal pain, weight loss, yellowing of the skin and eyes, anorexia (loss of appetite), nausea, changes in stool, and type 2 diabetes. Due to the late detection of the cancer, surgical removal of the tumor is only performed in less than 20% of patients. Thus, the aggressive nature of the disease, the lack of early markers and the lack of effective treatment options result in the lowest 5-year survival rate (3%- 7%) of all cancers in the United States (2–4) (Figure 2).

Figure 2: Age-specific 5-year relative survival (2000-2007) of pancreatic, bladder, and non-Hodgkin lymphoma cancers in both sexes in Europe.



Adapted from European cancer information system (ECIS) (4).

1.1.2. Risk factors

1.1.2.1. Non-modifiable risk factors:

Non-modifiable risk factors include type 2 diabetes (which starts at adulthood and is usually related to obesity), family history of pancreatic cancer, chronic pancreatitis (inflammation of the pancreas), ABO non-O blood group, and genetic mutations in *BRCA2*, *p16*, and other (5–7).

1.1.2.2. Modifiable risk factors:

Tobacco smoking is the main modifiable risk factor for pancreatic cancer and explains approximately 20% of the risk in a population where the prevalence of smoking is 30%. The pancreatic cancer risk increases with both smoking duration and intensity (8). Another well-established modifiable risk factor is obesity. Finally, red meat, processed meat, and heavy alcohol consumption may also increase the risk of pancreatic cancer (5–7).

1.2. Non-Hodgkin lymphoma

1.2.1. Epidemiology

In 2018, non-Hodgkin lymphoma was the 13th most common incident cancer and the 13th cause of cancer mortality worldwide (Figure 1). The aged-adjusted incidence rate of non-Hodgkin lymphoma was 12.5 and 8.1 per 100,000 inhabitants in North America and Europe, respectively, while in Asia was only 4.1 per 100,000 inhabitants (1). Incidence rates were higher in men than in women where the estimated male:female ratio in Europe was 1.5 to 1 in 2018 (1).

Non-Hodgkin lymphoma begins in the *lymphocytes* (white blood cells) which are part of the body's immune system, and its proliferation is located in a lymph gland. Non-Hodgkin lymphoma can start in any part of the body where lymph tissue can be found. However, the major sites are the lymph nodes.

Non-Hodgkin lymphoma cancers can be classified by type of white blood cell (T-cell or B-cell), where B-cell lymphoma is the predominant non-Hodgkin lymphoma (about 90% of all lymphomas). Among B-cell non-Hodgkin lymphoma, the most common

subtypes are diffuse large B-cell lymphoma, chronic lymphocytic leukemia / small lymphocytic lymphoma, follicular lymphoma, and multiple myeloma (9).

The mean age at diagnoses for non-Hodgkin lymphomas cancers is 60 (9). The 5-year relative survival rate ranges from 59% in Europe to 72% in the United States (4,10) (Figure 2).

1.2.2. Risk factors

1.2.2.1. Non-modifiable risk factors

Non-modifiable risk factors for non-Hodgkin lymphoma include several autoimmune diseases (such as rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus), organ transplantation, HIV-infection, and family history of blood malignancies. Further, hepatitis C virus has been associated with diffuse large B-cell lymphoma. Contrary, it has been observed that hay fever and any allergy (including plant, food, animal, dust, insect, or mold, but excluding drug allergies) are associated with lower non-Hodgkin lymphoma risk (11,12).

1.2.2.2. Modifiable risk factors

Certain occupations have been related to an increased risk of non-Hodgkin lymphoma, such as painter or farm worker. Obesity and tobacco smoking have also been associated with a higher risk of diffuse large B-cell lymphoma and follicular lymphoma, respectively. Higher socioeconomic status and recreational sun exposure are considered protective factors of non-Hodgkin lymphoma cancer. Finally, some evidence showed that alcohol intake might be associated with lower non-Hodgkin lymphoma risk (11,12).

1.3. Bladder cancer

1.3.1. Epidemiology

Bladder cancer was the 12th most common incident cancer and the 15th cause of cancer mortality worldwide (Figure 1) in 2018. Age-adjusted incident rates of bladder cancer were higher in high-income countries; being the highest in North America, followed by Europe and Australia/New Zealand. Rates in both North America and Europe were over 11 per 100,000 while in low-income countries were 3.6 per 100,000 inhabitants. In 2018, the estimated male:female ratio in Europe was 4.7 to 1 (1).

Urothelial carcinoma is the predominant bladder cancer type accounting for 95% of all cases in industrialized countries, other types of bladder cancers include squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and sarcoma (13).

Urothelial carcinomas can be classified based on their grade and stage as (14):

a. **Non-aggressive tumors:** these include pTa Grade 1 or 2 (where p indicates that the stage and grade have been based on pathology reports).
b. **Aggressive tumors:** these that are carcinoma *in situ* (CIS), or World Health Organization (WHO) Grade 3, or pT1 and higher.

More recent recommendations suggest to classify urothelial carcinomas by their tumor grade (15):

a. **Low-grade tumors**: these that are Grade 1.
b. **High-grade tumors:** these included Grades 2 and 3.

Urothelial carcinoma is considered a disease of the elderly being the mean age at diagnosis approximately 67 years (13). The 5-year survival rates depend on people diagnosed and ranges from 97% to 22%. The 5-years relative survival rate for all stages combined ranges from 68% in Europe to 76% in the United States. Moreover, the 5-year relative rate is higher in men (84%) than in women (75%) (4,13,16) (Figure 2).

1.3.2. Risk factors

1.3.2.1. Non-modifiable risk factors:

Non-modifiable risk factors comprise positive family history of urothelial carcinoma, genetic mutations in *p53* (tumor suppressor gene), *GSTM1* (Glutathione S-Transferase

Mu 1), and *NAT2* (N-Acetyltransferase 2) genes, and abnormalities in chromosome 9 (13,16).

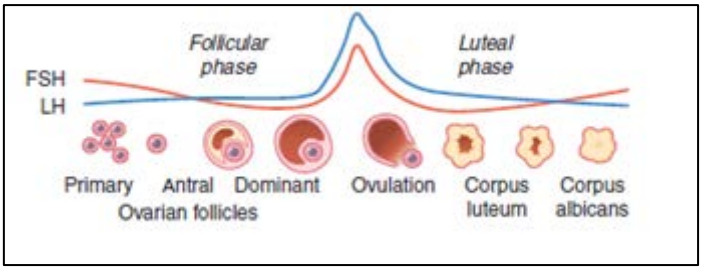
1.3.2.2. Modifiable risk factors:

Between 50-64% of urothelial carcinoma in men and 20-50% in women are attributable to tobacco use; and the risk increases with both intensity and duration of smoking (17). There is strong evidence that the ingestion of inorganic arsenic via drinking water is also related to an increased risk of urothelial carcinoma. Finally, people who work exposed to aromatic amines and dyes are at higher risk of developing urothelial carcinoma (13,16).

1.4. Endogenous hormones

From menarche to menopause, each endogenous hormone plays a role in the development and regulation of women's reproductive system. Periodically, hormonal levels fluctuate during the menstrual cycle, which starts the first day of menstruation and has a median duration of 28 days. The menstrual cycle could be divided into two phases: the follicular and the luteal phases (Figure 3).

Figure 3: Hormonal levels during the menstrual cycle



FSH: Follicle-stimulating hormone // LH: Luteinizing hormone. Adapted from Jameson 2010 (18)

1.4.1. Follicular phase

During the follicular phase, the follicle-stimulating hormone (FSH; hormone that regulates the development, growth, pubertal maturation, and reproductive processes of the body) achieves its highest level stimulating the secretion of estrogen. This increased level of estrogens rises the production of luteinizing hormone (LH; hormone that controls the ovulation); that achieves its highest peak approximately 34-36 hours previous to the ovulation. Estrogens levels gradually rise until day 14 when they dramatically drop and ovulation occurs. Then, the luteal phase begins (19).

1.4.2. Luteal phase

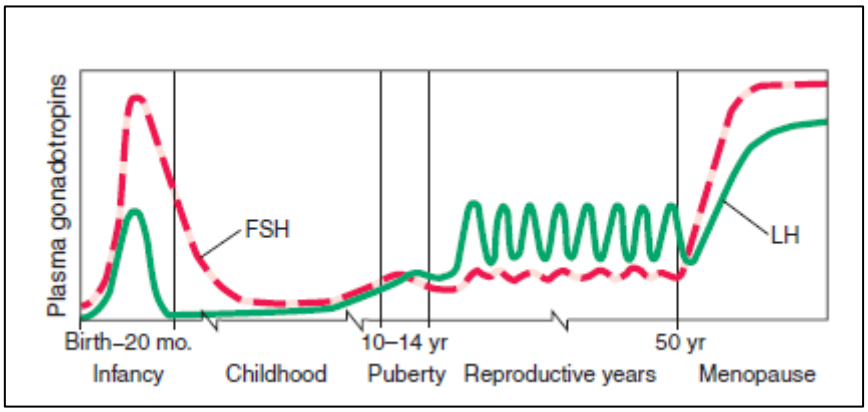
In the luteal phase, the predominant hormone is progesterone, which is responsible for preparing the endometrium for the implantation of the embryo. If pregnancy occurs its levels are maintained, if not its concentration decreases, and menstruation starts (19).

1.4.3. Menarche

During childhood FSH, LH, and estrogen levels are very low. Once puberty starts, FSH and LH levels increase gradually stimulating follicle maturation and estrogen synthesis in the ovaries (Figure 4). The increase of estradiol levels stimulates the growth of the endometrium and leads to menarche. Menarche is considered the first menstrual period in women and marks the beginning of a woman's fertile life. The firsts woman's cycles are usually irregular and anovulatory due to estradiol levels are still relatively low (20).

Age at menarche differs across countries and basically depends on both genetic (e.g. race/ethnicity) and environmental factors (e.g. social status, physical activity, diet, and overall health status). A decline in mean age at menarche was observed between the mid-19th and the mid-20th century, decreasing from 17 years to less than 14 years in the US and Western Europe. An improvement in nutrition could explain these secular trends (21). The International Collaboration for a Life Course Approach to Reproductive Health and Chronic Disease Events (InterLACE) consortium based on 23 studies (from 10 different countries) observed that the estimated mean age at menarche was 12.9 years, with a high heterogeneity between studies ranging from 12.5 to 13.6 years (22). Earlier ages at menarche (<12 years old) are related to a higher incidence of breast cancer and all causes of mortality (23).

Figure 4: Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels during woman life

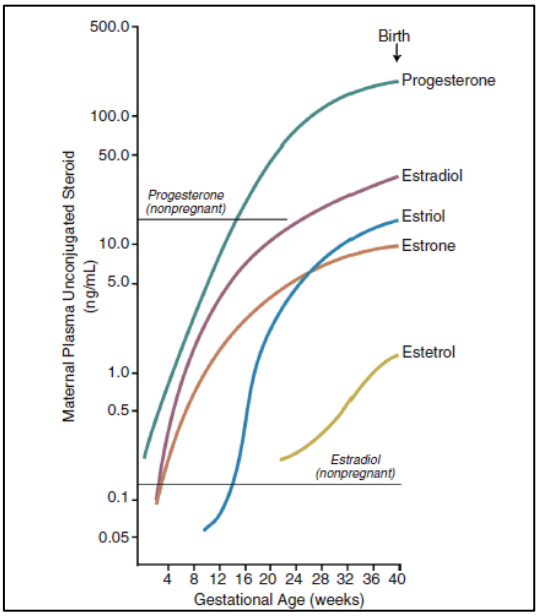


FSH: Follicle-stimulating hormone // LH: Luteinizing hormone. Adapted from Jameson 2010 (18)

1.4.4. Pregnancy and breastfeeding

Women experience several hormonal changes during pregnancy including an increase in estrogen and progesterone, which are 10 times higher at the end of pregnancy than in the luteal phase (24) (Figure 5). Further, during pregnancy prolactin levels progressively increase promoting breast growth in order to produce milk after birth (25). Prolactin serum levels reach 10 times pre-pregnancy levels during pregnancy; and during lactation, prolactin levels are about 30 times higher than pre-pregnancy levels.

Figure 5: Hormonal levels during pregnancy compared to hormonal levels in non-pregnant woman.



Adapted from Mesiano 2019 (26)

Age at first pregnancy, nulliparity, and the number of pregnancies vary depending on women's educational level. Later ages at first birth are related to higher educational level. Moreover, there is a higher proportion of nulliparous women associated with this group of highly educated women (22). Later age at pregnancy is also associated with a higher risk of miscarriage. Studies have shown an increased risk of miscarriage in women with more than 30 years; while after the age of 40 years, the risk of miscarriage increases rapidly (27–29). Miscarriage usually occurs before the 20th week.

Consistent epidemiological evidence has shown that nulliparous women are more likely to develop ovarian, endometrial, and breast cancer than women with children. Indeed, this protective effect is cumulative with each additional birth (30). Having the first birth after the age of 35 years increases the risk of developing breast cancer compare to women who have their birth before the age of 20 (31).

WHO recommends exclusively breastfeeding the first six months of the babies' life for the health of both mother and child, and a combination of breastfeeding with other foods and drinks for up to two years or longer (32). There is strong evidence that breastfeeding decreases the risk of breast cancer and limited evidence of a decreased

risk of ovarian cancer. During the breastfeeding period, women experience amenorrhea (absence of menstrual period) and infertility, which explain the lower risks of both breast and ovarian cancers (33).

1.4.5. Menopause

Menopause marks the end of a woman's fertile life and is an indicator of aging (34). Natural menopause is usually defined as 12 consecutive months without menstrual periods not associated with clinical causes. Before and during menopause, estrogen, progesterone, and androgens levels decrease, while FSH and LH levels increase (Figure 4). Anti-Müllerian hormone is used as an ovarian marker and as an indicator of a woman's ovarian reserve. Low levels of anti-Müllerian hormone can mean woman may have infertility problems or woman may undergo menopause (35,36).

Age at natural menopause differs across countries and in general occurs between 45 and 55 years (high-income countries from 50 to 52 years). Thus, if age at natural menopause occurs between 40 and 44 years is considered "early menopause" and if it occurs before 40 years "premature menopause" (37).

Later age at natural menopause is related to lower overall mortality and the risk of several diseases (e.g. osteoporosis and cardiovascular disease), but it is considered a risk factor for breast, endometrial and ovary cancers (38). Tobacco is the main risk factor of earlier age at natural menopause, where current smokers have a double risk of earlier age at natural menopause compared to nonsmokers (39). Some studies observed that high educational level, active lifestyle (only in former- or current-heavy smokers), high parity, oral contraceptive (OC) use, and alcohol intake are associated with later age at natural menopause (40). Inconsistent findings have been reported in relation to body mass index (BMI) (34). Influence of diet on age at natural menopause was also studied, but inconsistent results for vegetables, carbohydrates, and fiber intake were observed (37–39).

In epidemiological studies age at menarche and age at menopause are considered a proxy for endogenous hormone levels during fertile life. Furthermore, the number of pregnancies and breastfeeding are considered proxies of endogenous hormone changes during these phases of woman life.

1.4.6. Oophorectomy and hysterectomy

Oophorectomy is used to describe the surgical removal of one or both of the ovaries and it is used as a treatment of ovarian cancer, endometriosis, and pelvic inflammatory disease.

The term hysterectomy is used to describe the surgical removal of the uterus and cervix, while in partial hysterectomy it is only uterus is removed. Hysterectomy is the most common surgical procedure worldwide in gynecology worldwide. This surgery is used as a treatment for uterine, cervical and ovarian cancers, and non-cancerous uterine diseases, such as fibroids and hyperplasia. Almost 70% of American women will have fibroids during their fertile life (44). Fibroids can cause abnormal bleeding, elevated levels of pain, pelvic pressure, and emotional distress (44,45). Both fibroids and hyperplasia are related to high levels of estrogens; but, while elevated levels of progesterone are associated with fibroids, hyperplasia is associated with insufficient levels of progesterone (45,46).

Between 2000 and 2004, approximately 90% of hysterectomies in the United States were performed for a noncancerous cause. Further, the proportion of women undergoing both hysterectomy and oophorectomy at the same time was about 37% for women aged between 15 and 44 years, and 78% in those women older than 50 years (47).

Hysterectomy was related to decreasing levels of anti-Müllerian hormone (48) and elevated levels of FSH (49,50), causing that hysterectomized women with intact ovaries reach menopause about 4 years earlier than women without hysterectomy or oophorectomy. In addition, if at the same time of hysterectomy, one ovary is removed, premature ovarian function is over 4 years earlier compared to those women with hysterectomy and intact ovaries (49). Finally, women with one ovary removed enter menopause about two years earlier than women with intact ovaries (51).

The impact of hysterectomy with or without bilateral oophorectomy in hormone-related cancers is almost known. While hysterectomy with bilateral oophorectomy reduces the risk of breast and ovarian cancers; simple hysterectomy (with ovarian conservation) may increase the risk of ovarian cancer (52). Elevated risk of thyroid cancer was also related to hysterectomy with or without bilateral oophorectomy (53–55).

1.5. Exogenous hormones

The use of exogenous hormones, such as OC and menopausal hormone therapy (MHT), modifies hormone levels, and it depends on the type, dosage and route of administration of exogenous hormones.

The contraceptive pill is one of the main methods of contraception in Europe and the United States. It has been estimated that between 100 and 150 million women (about 10% of all women at reproductive age) women use contraceptive pills worldwide (56). OC preparations combined both estrogen (ethinyl estradiol) and progestogen and their combination have changed since they were introduced in the 1960s. Early OC preparations contained >50μg ethinyl estradiol, and these doses were reduced in the 1970s to 30μg. Current doses can be as low as 15μg. In OC users, the ovulation is suppressed because the progestin component inhibits LH secretion and/or the estrogenic component suppresses FSH secretion. So, during OC use women have lower plasma levels of natural estrogens and progesterone.

Until 2002, MHT was used to reduce menopause symptoms and to prevent osteoporosis and coronary heart diseases. Patterns of use of MHT type have changed during the past decades, with eras of estrogen alone or estrogen plus progestin. In 2002, the Women's Health Initiative (WHI), which included a clinical trial component and an observational study component, found that estrogen alone or estrogen plus progestin may not have the same biological effects. WHI assigned the treatment independently of the risk factors for the disease, which removes the potential confounding from observational studies. WHI observed that women who reported the combination of estrogen and progestin had a significantly increased risk of stroke, coronary heart diseases, breast cancer and a nonsignificantly decreased risk of endometrial cancer; however, taking estrogen alone did not show an increase of breast cancer in women with hysterectomy. Thus, estrogen-only MHT is usually recommended for women who have had a hysterectomy. These findings resulted in more than 50% reduction in MHT prescriptions (57,58).

In 2005, OCs and MHTs were classified as Group 1 carcinogens by the International Agency of Research of Cancer (IARC). Combined estrogen-progestin OCs have been associated with higher risks of breast, cervix, and liver cancers, but they have been inversely associated with endometrium and ovarian cancer risks. Estrogen-only MHT

has been directly associated with endometrial and ovarian cancers, and combined estrogen-progestogen MHT was also directly associated with breast cancers; while a decreased risk of endometrial cancer was observed for each day of use of progesterone (59,60).

1.6. Previous studies: endogenous hormones and exogenous hormones and cancers of the pancreas, non-Hodgkin lymphoma, and urothelial carcinoma

Animal studies examined the effect of sex hormones in relation to the growth of pancreatic, Non-Hodgkin lymphoma, and bladder cancers. Studies using castrated rats showed that the administration of sex steroids inhibits the development and growth of preneoplastic lesions of the pancreas (61,62). Furthermore, estrogen receptor (ER) β agonists have been reported to strongly inhibit the growth of lymphoma and leukemia cells in mice (63,64). Moreover, it has been shown that the incidence of bladder cancer was higher in male rats treated with testosterone supplementation than treated with estrogen supplementation (65). Finally, castration of male mice and pregnancy and/or lactation in female mice can decrease the growth of bladder cancer (66). It has been shown that ERα and ERβ are expressed in normal human peripheral blood cells and normal urothelial cells. Whereas, ERβ is the dominant receptor expressed in lymphoid neoplasms and urothelial carcinoma cells (64–67).

It has been described that reproductive hormones interact with the immune system and play a significant role in the etiology and pathophysiology of autoimmune diseases (71,72). Women produce a more vigorous cellular and humoral immune response than men. Thus, women suffer a higher incidence of autoimmune diseases (73), which are a known risk factor of non-Hodgkin lymphoma.

Motivated by these observations, several epidemiological studies have examined the possible associations between menstrual and reproductive factors, and hormone use, and the risks of pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma. At the beginning of this doctoral thesis results in relation to reproductive factors and exogenous hormone use with pancreatic cancer risk and non-Hodgkin lymphoma risk were weak and inconsistent (74–78). Comparing and summarizing previous evidence is not a simple task. Inconsistencies in results may arise from different categorization and reference categories of exposure variables, different adjustment or confounding

variables, and different study designs and target populations. In addition, many of previous studies were limited to statistical power to examine these associations.

The most consistent results were observed in relation to parity, age at natural menopause and urothelial carcinoma risk. Previous studies observed a lower risk of urothelial carcinoma with parity (79–83). An inverse trend between the number of births and urothelial carcinoma risk was only reported by one study (Weibull et al: Hazard Ratio (HR) for ≥3 vs. 1 full-term pregnancy: 0.76; 95% Confidence Interval (CI): 0.68-0.86) (80). Earlier age at menopause (natural or surgical) was associated with an increased risk of Urothelial carcinoma (83). In general, no associations between age at menarche, the use of OC, age at first full-term pregnancy, and breastfeeding and urothelial carcinoma have been observed (79–89). A meta-analysis by MHT formulation, based on four studies, showed a possible reduced risk of urothelial carcinoma in women who used estrogen plus progestin MHT compared to never users of MHT (79). Nevertheless, in the WHI no such association was observed (81).

2. RATIONALE

As shown in the previous chapter, menopause is an inflexion point in women's health. Decreasing levels of estrogen, progesterone, and androgens are related to several diseases, such as osteoporosis and cardiovascular diseases. However, the risk of hormone-related cancers decreases after menopause. Thus, studying modifiable risk factors that are sensible to modify age at natural menopause are of special interest for women's health.

The most established modifiable factor is tobacco smoking. Several European studies observed some associations between educational level, physical activity, and OC use. Inconsistent results were observed in relation to diet. Finally, previous studies observed a reduced risk of earlier age at natural menopause with alcohol intake. It has been shown that age at natural menopause varies across countries and few studies have included Spanish participants. Therefore, this doctoral thesis has been focused on Caucasian women, particularly Spanish women.

Women are exposed to endogenous hormonal changes during their life. Additionally, exogenous hormones for both contraception and minimizing menopause symptoms are taken frequently. Thus, clarifying the role of endogenous and exogenous hormones in women health is of great interest to the scientific community, especially the role of exogenous hormones.

Menstrual factors, reproductive factors and history of exogenous hormones are well-established risk factors of hormone-related cancers (such as breast, endometrial, and ovarian tumors). In general, earlier age at menarche was related to breast cancer and ovarian cancers. Nulliparity and late age at menopause increase the risk of developing hormone-related cancers. OC therapy increases the risk of breast cancer, but it protects against ovarian and endometrial cancers. Finally, the use of MHT has been shown to increase the risk of these three cancers (90). Nevertheless, the influence of menstrual factors, reproductive factors and exogenous hormones is still unclear in non-hormone related cancers with a different incidence in men and women. Pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma are more common in men than women.

As shown in the previous chapter, the role of endogenous and exogenous hormones is not clear in relation to pancreatic cancer risk and non-Hodgkin lymphoma risk yet. Contrary, some evidence has been observed in relation to endogenous and exogenous hormones and the risk of urothelial carcinoma. Several studies observed the relation of reduced risks of urothelial carcinoma with some reproductive factors, such as parity, independent of the number of births, and OC use. A possible reduction in urothelial carcinoma risk in women who used estrogen plus progestin has been found.

The work of this doctoral thesis will contribute to increasing the existing scientific evidence for understanding the role of modifiable factors in age at natural menopause. Moreover, this thesis will help to figure out whether endogenous and exogenous hormones influence the risks of the pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma.

3. HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

Hypothesis 1: Lifestyle factors (including educational level, BMI, physical activity, and tobacco), diet, and menstrual and reproductive history, and OC use influence on the age of natural menopause.

1. Smoking habits, sedentary lifestyle, and low educational level increase the risk of an earlier age at natural menopause.
2. Healthy dietary habits, and foods and nutrients with known estrogenic potential (soy, fiber, fats, vitamin D, polyphenols, and nuts) are protective against an earlier age at natural menopause.
3. Parity and OC use are protective against an earlier age at natural menopause.

Hypothesis 2: Menstrual factors, reproductive history, and exogenous hormone use are protective factors of pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma.

1. A greater exposure to female sex hormones (i.e., earlier age at menarche, later age at menopause, parity, and use of exogenous hormones) is a protective factor against pancreatic cancer.
2. Parity, later age at menopause and use of exogenous hormones are protective factors against urothelial carcinoma.
3. Parity and exogenous hormone use are protective factors against non-Hodgkin lymphoma.

3.2. Objectives

Objective 1: To evaluate the role of lifestyle factors, diet, and menstrual and reproductive history, and oral contraceptive use in age at natural menopause.

Objective 2: To evaluate the role of menstrual factors, reproductive history, and exogenous hormones and the risk of pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma.

4. METHODS

This doctoral thesis is based on the results of two large epidemiological studies:

- **PanC4** (Pancreatic Cancer Case-Control Consortium), an international consortium of case-control studies of pancreatic cancer including studies from around the world (i.e. North America, Western and Eastern Europe, Asia, and the Middle East).
- **EPIC** (European Prospective Investigation into Cancer and Nutrition), a multicenter cohort study, run in 23 centers from 10 European countries.

The aims and methods of these two studies are summarized in the next sections:

4.1. PanC4 study

The PanC4 study is an international consortium of case-control studies of pancreatic cancer. The aim of PanC4 is to increase the knowledge about the risk factors and preventive factors for pancreatic cancer, including environmental, lifestyle, dietary, genetic, and social factors. Nowadays, 26 case-control studies comprise the PanC4 consortium. At the beginning of this doctoral thesis, from these 26 case-control studies, eleven provided information on menstrual and reproductive factors and use of exogenous hormones. At least all included studies in this doctoral thesis were able to provide information on ages at menarche and menopause. Of these, four studies were conducted in North America, four in Europe, two in China, and one was a multicenter study from Canada, Europe, and Australia.

The total number of women available in the combined dataset was 2,838 participants with pancreatic cancer and 4,748 controls. A summary description of the general characteristics of the individual studies is presented in Table 1. Briefly, controls from Toronto, the University of California, San Francisco (UCSF), Central Europe study, China (Shanghai I and II), and the Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH) were from the general population of study areas. The MD Anderson Cancer Center (MDACC) study and the Memorial Sloan Kettering Cancer Center (MSKCC) study selected controls from hospital visitors genetically unrelated to cases. Hospital-based controls were selected in Milan and Italy. Finally, Greece included both hospital visitors and hospital-based controls. The SEARCH study, Toronto, and Shanghai-I studies

included proxy responders, accounting for 14.5% of the case and 4.4% of the control women.

Table 1: Summary description of individual studies included in the International Pancreatic Cancer Case-Control Consortium (PanC4) on pancreatic cancer and reproductive factors and exogenous hormones use.

	Cases n (%)	Controls n (%)	Source of controls	Type of interview
Pancreatic cancer	2838 (37.4)	4748 (62.6)		
Study				
North America				
MDACC (2004-2008)	257 (9.1)	288 (6.1)	Hospital visitors	Direct
MSKCC (2003-2010)	424 (14.9)	242 (5.1)	Hospital visitors	Direct
Toronto (2003-2006)	217 (7.7) (6.5% proxies)	134 (2.8)	Population	Direct/ Proxy (4%)
UCSF (1995-1999)	237 (8.4)	818 (17.2)	Population	Direct
Europe				
Central Europe study; Poland, Czech Republic, Slovakia (2004-2009)	361 (12.7)	435 (9.2)	Population	Direct
Greece (1990-1992)	66 (2.3)	132 (2.8)	Hospital, Hospital visitors	Direct
Milan (1982-1999)	133 (4.7)	409 (8.6)	Hospital	Direct
Italy (1991-2008)	148 (5.2)	304 (6.4)	Hospital	Direct
China				
Shanghai-I (1990-1993)	187 (6.6) (29.4% proxies)	701 (14.8) (5.6% proxies)	Population	Direct / Proxy (10.6%)
Shanghai-II (2006-2011)	445 (15.7)	464 (9.8)	Population	Direct
International				
SEARCH; Canada, The Netherlands, Poland, Australia (1983-1989)	363 (12.8) (58.4% proxies)	821 (17.3) (16.81% proxies)	Population	Direct / Proxy (29.6%)

MDACC indicates MD Anderson Cancer Center; MSKCC, Memorial Sloan Kettering Cancer Center; SEARCH, Surveillance of Environmental Aspects Related to Cancer in Humans; UCSF, University of California, San Francisco. Adapted from Lujan-Barroso et al (91).

4.1.1. Core variables

Individual data on sociodemographic factors, anthropometric measures, tobacco smoking, alcohol consumption, and history of pancreatitis was collected in each study. History of diabetes was based on self-reported information from a questionnaire including age at first diagnosis. The original datasets were restructured either by the study investigators or central coordinators using a uniform format for data harmonization. Information on alcohol was not available in the MSKCC study, and history of pancreatitis was not collected in the Italian study.

4.1.2. Menstrual and reproductive history, and use of exogenous hormones variables

The principal investigators of the included studies on this doctoral thesis shared with us their individual datasets with information on menstrual and reproductive history, and use of exogenous hormones. Collected data about menstrual and reproductive factors and exogenous hormone use was generally similar across all studies; however, full harmonization of the data was performed with the collaboration of the investigators of each study. The information available for each study is tabulated in Table 2.

4.1.3. Statistical analyses

To estimate pooled odd ratios (ORs) and 95% CI for the eleven case-control studies on menstrual and reproductive factors, and exogenous hormones and the risk of pancreatic cancer two-stage statistical models were used. At the first stage, study-specific logistic regression models were performed in order to assess the association between each exposure factor and pancreatic cancer risk (92). All models were adjusted for age (<45, 45-49,50-54, 55-59, 60-64, 65-69, 70-75, ≥75 years), education (≤8th grade, 9th-11th grade, 12th grade or high school graduates, some college or college graduate, ≥ 1 year of graduate school), usual BMI (<20, 20-<25, 25-<30, ≥30 kg/m^2), history of non-gestational diabetes mellitus, cigarette smoking (never-smokers, current smokers <20 cigarettes/day, current smokers ≥20 cigarettes/day, former smokers <10 years after quitting, former smokers ≥10 years after quitting), alcohol (no info available, 0-<1, 1-<4, ≥4 drinks/day), race (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, American Indian/Alaskan native) and center (for multicentric

studies). History of pancreatitis was not included as an adjustment covariate in the present analysis because some of the studies did not have controls with this exposure. At the second step, pooled effect estimates across studies were calculated using random effect meta-analysis (93). To evaluate heterogeneity across studies, we calculated the χ^2 statistic and the index I^2 (that represents the proportion of total variation in the estimates of the exposure factor effect that is due to heterogeneity between studies) (94). In those models with a significant heterogeneity between studies, Galbraith plots were used to examine sources of heterogeneity (95), and sensitivity analyses excluding the identified study/studies were performed to evaluate study influence on pooled ORs.

Table 2: Menstrual and reproductive history, and use of exogenous hormones provided by each study and center.

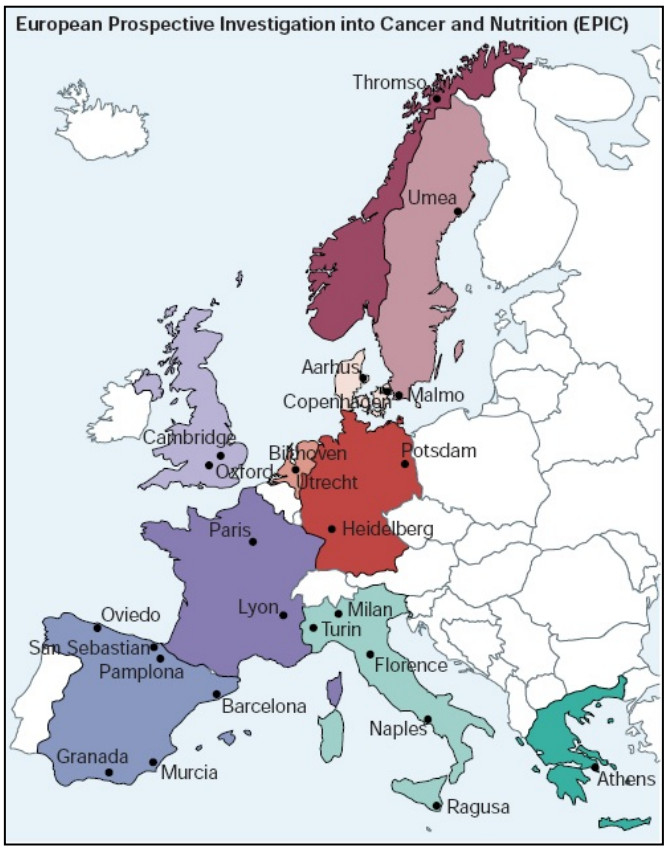
	PanC4	EPIC study
Age at menarche	All studies have information.	All centers have information.
Cumulative duration of menstrual cycling	Information not available in MDACC, SEARCH, Toronto and Greek studies.	All centers have information.
Use of OC	Information not available in the Greek study.	All centers have information.
Duration OC use, year	Information not available in the Greek study.	Information not available in Bilthoven.
Menopausal status	All studies have information.	All centers have information.
Type of menopause	Information not available in Shanghai-I study.	All centers have information.
Age at menopause, years	All studies have information.	All centers have information.
Use of MHT	Information not available in the Greek study.	All centers have information.
Duration MHT use, years	Information not available in the Greek study.	All centers have information.
Type of MHT	Not available.	Available in France, Italy, Spain, United Kingdom, The Netherlands, Germany, Denmark, and Norway.
Oophorectomy	Information not available in the Milan, Greek, Shanghai-I and Shanghai-II studies.	Information not available in Malmö.
Hysterectomy	Information not available in the Milan, Greek, Shanghai-I and Shanghai-II studies.	Information not available in Malmö.
Parity	Information not available in the MDACC study.	All centers have information.
Number of full-term pregnancies	Information not available in the MDACC study.	Information not available in Bilthoven.
Age at first full-term pregnancy	Information not available in the MDACC study.	All centers have information.
Breastfeeding	Not available.	Information not available in Bilthoven and Umeå.
Duration of breastfeeding, all pregnancies	Not available.	Information not available in Bilthoven and Umeå.
Induced abortions	Information not available in the MDACC study.	Information not available in Bilthoven, Malmö, Umeå, and Norway.
Spontaneous abortions	Information not available in the MDACC study.	Information not available in Bilthoven, Umeå, and Norway.
Fertility problems	Not available.	Available in France, Italy, Spain, United Kingdom, Utrecht, Greece, and Germany.

MDACC indicates MD Anderson Cancer Center; MSKCC, Memorial Sloan Kettering Cancer Center; SEARCH, Surveillance of Environmental Aspects Related to Cancer in Humans; UCSF, University of California, San Francisco.

4.2. EPIC study

The EPIC study is an ongoing multicenter prospective cohort study that recruited volunteer participants from 23 centers located in ten European countries including Sweden, Denmark, Norway, the Netherlands, United Kingdom, France, Germany, Spain, Italy, and Greece (Figure 6). The aim of the study is to evaluate the association between diet and life-style habits and the risk of cancer and other chronic diseases. It is based on healthy adults which their health status have been followed over time.

Figure 6: Location of all EPIC collaboration centers



A total of 521,324 participants were recruited between 1992 and 2000, mostly aged 35-70 years. The majority of the participants were invited from the general adult population; however, there were several exceptions. The French cohort was based on members of the health insurance for teachers; participants of the Italian and Spanish cohorts included members of local blood donor associations; Utrecht (The Netherlands) and Florence (Italy) cohorts invited women from a local population-based breast cancer screening program. In Oxford (United Kingdom) half of the cohort was recruited among subjects who did not eat meat (including vegans, lacto-ovo vegetarians and fish

consumers). Finally, France, Norway, Utrecht (The Netherlands), and Naples (Italy) cohorts only recruited women (96).

Information on diet, lifestyle, and anthropometric measurements was collected only at baseline. Questionnaires specific to women were used to collect information on menstrual factors, reproductive history, and use of exogenous hormones.

4.2.1. Dietary intake assessments

Country-specific validated questionnaires, which included a complete list of different types of foods, were used to collect dietary information. In northern Italy, The Netherlands, Germany and Greece self-administrated quantitative dietary questionnaires containing up to 260 food items and estimating individual average portions systematically were used. Self-administrated quantitative questionnaires structured by meals were used in Spain, France and Ragusa (South Italy). While in Spain and Ragusa, dietary data was collected in face-to-face interviews; in France, questionnaires were self-reported. Denmark, Norway, Naples (Italy) and Umeå (Sweden) used semi-quantitative food-frequency questionnaires. Finally, combined dietary methods were used in the United Kingdom and Malmö (Sweden) cohorts. Energy and nutrient intakes were estimated using the standardized EPIC Nutrient Database (ENDB) (96).

To assess objective 1, adherence to the Mediterranean diet was analyzed using the adapted relative Mediterranean diet (arMED) index. The arMED index consists of a 16-point scale that incorporates eight key components of the Mediterranean diet. For the six components presumed to fit a Mediterranean diet: fruits (including nuts and seeds), vegetables (excluding potatoes), legumes, fish (fresh or frozen, excluding fish products and preserved fish), olive oil and cereals, a score of 0-2 was assigned to tertiles of intake. The scoring was inverted for the components presumed to not fit a Mediterranean diet: meat and dairy products. The points were summed to define the arMED score that ranges from 0 (no adherence) to 16 (maximum adherence) and represents the level of adherence to the Mediterranean diet (97). Alcohol was not included in the arMED index since it is a potential risk factor for a later age at natural menopause. Information on past alcohol intake was assessed as average number of glasses of beverage consumed per week at ages 20, 30, 40, and 50 years. The weighted average intake with weights equal to the amount of time of exposure to alcohol during decades was calculated to determine average lifetime alcohol intake (98).

4.2.2. Lifestyle information assessments

Information on lifestyle factors (including smoking history, physical activity, educational level, current and past occupation which might have led to exposure to carcinogens, and alcohol drinking), health factors (history of previous illness, disorders or surgical interventions), and menstrual and reproductive history, and use of exogenous hormones (OC and MHT) were collected at baseline. The information available for each EPIC center regarding menstrual and reproductive factors, and use of exogenous hormones is shown in Table 2.

The questionnaire on physical activity consisted of three different domains of physical activity: occupational, recreational, and household activity. Since the occupational physical activity data did not include the duration and the frequency of the activity, it was not possible to combine all activity collected into one overall variable expressed as MET-hours/week. Thus, we used physical activity level based on the validated Cambridge Physical Activity Index, where work activity and leisure time physical activity were combined (Table 3) (99).

Table 3: Classification of physical activity according to the Cambridge Physical Activity Index:

	Leisure time physical activity (Duration of sport and cycling in hours/week)			
Work activity	**No**	**≤ 3.5**	**>3.5 and ≤ 7.0**	**> 7.0**
Sedentary	Inactive	Moderately inactive	Moderately active	Active
Standing	Moderately inactive	Moderately active	Active	Active
Manual	Moderately active	Active	Active	Active
Heavy manual	Active	Active	Active	Active

Finally, baseline anthropometry information (including height, weight, and waist and hip circumference) was measured by the interviewers in all centers except in France, Oxford and Norway where weight and height data were self-reported. In Oxford, self-reported waist and hip circumference measurements were also collected. Umeå (Sweden) and France did not collect data on waist and hip circumference. Table 4 summarizes the non-dietary information provided by each EPIC center.

Table 4: Non-dietary information provided by each center

	EPIC study
Anthropometry	All centers except Umeå and Tromsø have either self-reported (France and part of the United Kingdom) or measured information on weight, hip circumference and waist circumference. Umeå and Tromsø information is available on weight and height.
Physical activity	All core centers have information on type of physical activity at work, physical exercise gardening, housework, and number of stairs climbed per day. Of the associated participants, the Danish centers have complete information, the center in Malmö has the majority of information, and the center in Umea information is limited on type of physical activity at work.
Tobacco smoking	All centers have information on smoking status at baseline and number of cigarettes smoked.
Alcohol consumption	The core centers have information on past amount of wine, beer/cider, fortified wine, and spirit/liquor consumed. In addition, for Cambridge, Bilthoven, and Greece, information on current levels of consumption for each of these types of alcohol is available as non-dietary variable. Of the associated participants, the Danish and Naples centers have complete information. Malmö and Norway center have information on current alcohol consumption only. No information on past alcohol consumption is available in Umea. For all EPIC centers, additional information on current alcohol consumption is available from the dietary questionnaires.
Occupational history	Information available in centers from Italy, Spain, Cambridge, Greece, Germany, and Denmark. The Norwegian center has information on current occupation.
Socio-economic status	All centers have information on highest school level achieved.
Previous illnesses	All centers have information on heart disease and diabetes, while the majority of centers have information on stroke, hypertension, hyperlipidemia, gall stones, polyps of the large bowl, hysterectomy, oophorectomy, and breast surgery, and information on age of onset of each of these events.

Core centers include centers in France, Italy (except Naples), Spain, United Kingdom, The Netherlands, Greece, and Germany. The associated participants include centers in Sweden, Denmark, Norway, and Naples. Adapted from Riboli 2002 (96)

4.2.3. Identification of menopausal cases during the follow-up

Updated information on menopausal status and age at menopause was collected after a median follow-up of three years in the EPIC-Spanish cohort. A total of 15,659 women completed a short follow-up questionnaire, including information on reproductive history.

For those women who were identified as premenopausal at recruitment, age at natural menopause was defined as the age at the last menstrual period (years) for those women without menstrual periods for at least 12 consecutive months. Women who reported bilateral oophorectomy or hysterectomy previous to natural menopause or use of MHT without recoding age at menopause (natural menopause can be masked by bleeding caused by the therapy) were excluded for the analyses of the objective 1. Furthermore, women with possible underlying menopausal symptoms (OC users older than 40 years) were also excluded.

4.2.4. Ascertainment of cancer cases and vital status

Incident cancer cases and data on vital status (cause and date of the death) were identified through population registries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden, and United Kingdom) and active follow-up, including use of health insurance records, hospital registries, and direct contacts with participants or next-of-kin (France, Germany, and Greece). For this doctoral thesis, the follow-up for cancer cases was completed between December 2011 and December 2013, depending on the center.

Lymphoid neoplasms were initially classified according to the International Classification of Diseases for Oncology, Second Edition (ICD-O-2), and were later recoded according to the International Classification of Diseases for Oncology, Third Edition, from the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues (100). The conversion was made using an algorithm available on the Surveillance, Epidemiology, and End Results web page (http://www.seer.cancer.gov/) and involved a pathology expert and local expertise from participating EPIC centers. Cases with ICD-O-2 codes that could not be translated unequivocally into a lymphoid neoplasm according to the WHO classification system were categorized as “lymphoid neoplasm, unclassified”. The classification was further revised by participating EPIC centers using the InterLymph PathologyWorking Group classification, which is based on the 2008 WHO classification (100).

Bladder cancer diagnoses were coded according to the ICD-O-3 as C67 based on ICD-O-3 and cases with morphology codes 812*–813* were identified as urothelial carcinoma cases (14). Only urothelial carcinoma was included in the present analyses; since it approximately represents 95% of all bladder cancers in industrialized countries. Definitions and classifications of urothelial carcinoma subtype are heterogeneous in the literature. In previous EPIC studies, urothelial carcinoma was classified by pathology reports as aggressive (pT1 and higher or carcinoma in situ (CIS) or WHO Grade 3), and non-aggressive (pTa Grade 1 and 2) (14). We also analyzed urothelial carcinoma by tumor grade using WHO-definition: Grades 2 and 3 as “high-grade”, and Grade 1 as “low-grade” (15).

4.2.5. Statistical analyses

To evaluate associations between exposure factors and age at natural menopause, non-Hodgkin lymphoma, or urothelial carcinoma, Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95%CI. Ordinal variables were scored from 0 to their maximum value and trend tests were calculated on these scores. Age was used as the time scale, with age at recruitment as the entry time, and age at the date of age at natural menopause, non-Hodgkin lymphoma, urothelial carcinoma, or end of follow-up (whichever came first) as the exit time. All Cox proportional hazard regression models were stratified by center, and age at recruitment (1-year categories) and adjusted for each respective outcome confounders. Stratified models by centers allowed us to give each center its own baseline hazard, thus the variation in menstrual and reproductive history, hormone use, and cancer patterns across centers are included in the model. Further, stratified by age provided left truncation of the data (the risk of developing the outcomes of interest is only included during the follow-up). Finally, these stratified models assume proportional hazard between the centers.

Additional analyses to evaluate the heterogeneity of the risk between non-Hodgkin lymphoma subtypes, and urothelial carcinoma tumor aggressiveness and urothelial carcinoma tumor grade were performed by means of the Wald test statistic using the %*SUBTYPE* SAS macro (101).

A brief summary of the material and methods used in the articles can be found in the Table 5.

Table 5: Summary of main methodological issues by study and publication

	Publication 1	Publication 2	Publication 3	Publication 4
Design	Multicenter Spanish cohort	Pooled analysis of case-control studies	Multicenter European cohort	
Outcome	Age at natural menopause	Pancreatic cancer	Non-Hodgkin lymphoma	Urothelial Carcinoma
Study	EPIC-Spain	PanC4	EPIC	EPIC
Number of cases	1,166	2,838	1,427	529
Total number of participants	12,562	7,586	343,458	333,919
Exposures	Lifestyle, diet, reproductive history, and OC use	Menstrual and Reproductive factors and exogenous hormone		
Statistical methods	Cox proportional hazard models	Logistics regressions with random effects meta-analysis	Cox proportional hazard models	
Statistical software	SAS v9.4	SAS v9.2.1 Stata v15.0	SAS v9.4	SAS v9.4

EPIC: European Prospective Investigation into Cancer and Nutrition // PanC4: Pancreatic Cancer Case-Control Consortium // OC: oral contraceptive

5. ETHICAL ASPECTS

All participants who accepted to be involved in any of the studies included in both the PanC4 and EPIC projects signed an informed consent form. Moreover, all data derived from the questionnaires was used anonymously for the study. The EPIC cohort and PanC4 studies were approved by center-based ethical committees in their respective countries, and EPIC cohort was also globally approved by the IARC ethical committee. This doctoral thesis proposal has been approved by the ethical committee of the Bellvitge Hospital (Comité Ético de Investigación Clínica sobre Proyectos de Investigación – CEIC) (Annex 1).

6. RESULTS

The present chapter introduces the 4 publications presented in this doctoral thesis, three of them have been published in international journals (Table 6).

Table 6: Impact factor, category, and journal rank of the articles presented in this doctoral thesis

Authors and title	Journal & publication date	IF	Category - Journal rank
Lujan-Barroso L, Gibert K, Obón-Santacana M, Dolores Chirlaque M, Sánchez MJ, *et al.* The influence of lifestyle, diet, and reproductive history on age at natural menopause in Spain: Analysis from the EPIC-Spain sub-cohort.	*Am J Hum Biol* (2018) 30(6) e23181	1.438	Anthropology - Q2
Lujan-Barroso L, Zhang W, Gao Y-T, Yu H, Bracci PM, Baghurst PA, *et al.* Menstrual and Reproductive factors, hormone use, and risk of pancreatic cancer case-control consortium (PanC4).	*Pancreas* (2016); 45 (10): 1401-10	2.967	Gastroenterology & Hepatology – Q2
Costas L, **Lujan-Barroso L,** Benavente Y, Allen NE, Amiano P, Ardanaz E, *et al.* Reproductive Factors, Exogenous Hormone Use, and Risk of B-Cell Non-Hodgkin Lymphoma in a Cohort of Women from the European Prospective Investigation Into Cancer and Nutrition.	*Am J Epidemiol* (2018); 188 (2): 274-81	4.473	Public, environmental & occupational health - Q1
Lujan-Barroso L, Botteri E, Caini S, Ljungberg B, Roswall N, Tjønneland A, *et al.* Menstrual factors, reproductive history, hormone use, and Urothelial carcinoma risk: A prospective study in the EPIC cohort	British Journal of Cancer; under-review	5.416	Oncology – Q1

IF: Impact factor

6.1. Summary of results

6.1.1. Lifestyle, diet, and reproductive history in earlier age at natural menopause risk

In total 12,562 pre-menopausal Spanish women were included in the analysis. After a median of 3 years of follow-up, 1,166 became postmenopausal. The median age at natural menopause in the EPIC-Spain cohort was 51 years. Earlier age at natural menopause was associated with current smokers at baseline (HR: 1.29; 95%CI: 1.08- 1.55). A later age at natural menopause was observed in women with irregular menses the first 10 years after menarche (HR: 0.71; 95%CI: 0.56- 0.91) and in women with more than 1 full-term pregnancies ($HR_{\geq 4 vs 0}$: 0.74 95%CI: 0.56- 0.94; *P* for trend = 0.027). Finally, among OC users, later age at natural menopause was observed in women who started treatment between the ages of 25 and 30 years compared to those who started after the age of 31 years (HR: 0.72; 95%CI: 0.58- 0.89). No associations were observed for dietary variables and the remaining lifestyle and reproductive history factors.

6.1.2. Menstrual factors, reproductive history, and exogenous hormones in pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma risks

6.1.2.1. Pancreatic cancer

Eleven case-control studies within the PanC4 consortium took part in the analysis, including in total 2,838 case and 4,748 control women. An inverse association was observed between pancreatic cancer and women who reported having had hysterectomy (OR: 0.78; 95%CI: 0.67- 0.91), remaining significant in postmenopausal women and in never-smoking women. A model including the join effect for MHT and hysterectomy showed a significant inverse association with pancreatic cancer in women who reported having had hysterectomy with MHT use (OR: 0.64; 95%CI: 0.48- 0.84). No associations were observed for menstrual and reproductive factors and OC use and pancreatic cancer.

6.1.2.2. Non-Hodgkin lymphoma

A total of 343,458 women, from the EPIC study, were included for the analysis. After a median of 15 years of follow-up of 1,427 incident cases of non-Hodgkin lymphoma were identified. Overall, non-statistically significant associations between parity, age at first birth, breastfeeding, OC use, MHT use and the risk of non-Hodgkin lymphoma was observed. Women who had undergone surgical menopause were at higher risk of non-Hodgkin lymphoma compare to natural menopause (HR: 1.51; 95%CI: 1.17- 1.94). Either hysterectomy or oophorectomy alone were associated with non-Hodgkin lymphoma risk, while women with hysterectomy plus oophorectomy (especially bilateral oophorectomy) showed a higher risk of non-Hodgkin lymphoma (HR: 1.26; 95%CI: 1.01- 1.56).

6.1.2.3. Urothelial carcinoma

After exclusions, a total of 333,919 women, from the EPIC study, were included for the analysis. After a median follow-up time of 15 years, 529 urothelial carcinoma cases were identified. Elevated and statistically significant HR for urothelial carcinoma was observed for peri-/postmenopausal (natural or surgical) MHT-users compared to peri-/postmenopausal women never-users (HR: 1.27; 95%CI: 1.03- 1.57). Statistically significant inverse associations between number of full-term pregnancies and UC risk were observed (HR_{3vs1}: 0.70; 95%CI, 0.52- 0.94; $HR_{\geq 5vs1}$: 0.48; 95%CI, 0.25-0.90; *P* for trend in parous women only 0.010). Age at menarche, cumulative duration of menstrual cycling, history and duration of OC use, age at first birth, breastfeeding, abortions (spontaneous and induced), age at natural menopause, oophorectomy, and hysterectomy showed no association with urothelial carcinoma risk.

PAPER – I

The influence of lifestyle, diet, and reproductive history on age at natural menopause in Spain: Analysis from the EPIC-Spain sub-cohort

Lujan-Barroso L, Gibert K, Obón-Santacana M, Dolores Chirlaque M, Sánchez MJ, Larrañaga N, Barricarte A, Quirós JR, Salamanca-Fernández E, Colorado-Yohar S, Gómez-Pozo B, Agudo A, Duell EJ.

Am J Hum Biol (2018) 30(6) e23181

Received: 18 October 2017 | Revised: 6 July 2018 | Accepted: 12 August 2018

DOI: 10.1002/ajhb.23181

WILEY American Journal of Human Biology

ORIGINAL RESEARCH ARTICLE

The influence of lifestyle, diet, and reproductive history on age at natural menopause in Spain: Analysis from the EPIC-Spain sub-cohort

Leila Lujan-Barroso | Karina Gibert | Mireia Obón-Santacana | María Dolores Chirlaque | María-Jose Sánchez | Nerea Larrañaga | Aurelio Barricarte | Jose Ramón Quirós | Elena Salamanca-Fernández | Sandra Colorado-Yohar | Basilio Gómez-Pozo | Antonio Agudo | Eric J Duell

Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain

Correspondence
Leila Luján-Barroso, MSC Unit of Nutrition and Cancer Cancer Epidemiology Research Program Catalan Institute of Oncology (ICO-IDIBELL) Avda Gran Via 199-203 08908 L'Hospitalet de Llobregat Barcelona, Spain.
Email: llujan@iconcologia.net

Abstract

Objective: To determinate the role of lifestyle factors, recent diet, menstrual factors, and reproductive history in age at natural menopause in adult Spanish women.

Methods: In total, 12 562 pre-menopausal women were available for analysis from the EPIC-Spain sub-cohort. Women were recruited between 1992 and 1996 in five regions of Spain (Asturias, Granada, Murcia, Navarra, and San Sebastian) and, for these analyses, were followed for 3 years. Questionnaires on diet, lifestyle, anthropometric measurements, and reproductive and exogenous hormones history were collected at baseline. Menopause status was updated at a median of 3 years of follow-up.

Results: After a median of 3 years of follow-up 1166 women became postmenopausal. An earlier age at menopause was observed in current smokers (HR: 1.29; 95%CI 1.08-1.55) and in non-users of oral contraceptives (HR: 1.32; 95%CI 1.01-1.57). A later age at menopause was observed in women with irregular menses (HR: 0.71; 95%CI 0.56-0.91) and in women with a higher number of pregnancies (HR: 0.74; 95%CI 0.56-0.94).

Conclusions: Our results confirm that women who smoked had an earlier age at natural menopause, while use of oral contraceptives, higher number of pregnancies, and irregularity of menses were associated with a prolonged reproductive lifespan. No associations were observed for dietary habits assessed after the age of 40 years.

KEYWORDS

age at menopause, diet, lifestyle, menopause, reproductive history

1 | INTRODUCTION

Menopause marks the end of a woman's fertile life, and is an indicator of aging (Gold, 2011). Natural menopause is usually defined as 12 consecutive months without menstrual periods not associated with clinical causes (NAMS (The North American Menopasue Society), 2017). Before and during menopause, women experience changes in endogenous sex hormones such as decreasing levels of estrogen, progesterone, and androgens. However, follicle-stimulating (FSH) and luteinizing (LH) hormone levels increase at menopause (NAMS, 2017). Thus, menopause can be considered an inflection point in a woman's overall health. Later age at menopause reduces overall mortality and the risk of several

diseases (eg, osteoporosis and cardiovascular diseases). However, later age at natural menopause (ANM) is also considered a risk factor for cancers of the breast, endometrium and ovary (Daan & Fauser, 2015). Thus, a more complete understanding of determinants of natural menopause is of clinical interest.

ANM varies across different countries, and in general occurs between 45 and 55 years. "Early menopause" occurs between 40 and 44 years, and before 40 years it is considered "premature menopause" (Dratva et al., 2009; NAMS, 2017). In developed countries, ANM typically ranges from 50 to 52 years (Gold, 2011). Further, some studies in women of European descent observed later ANM with higher educational level (Kaczmarek, 2007; Martin et al., 2006; Stepaniak et al., 2013). A previous meta-analysis (Sun et al., 2012) observed that current smokers presented an approximate twofold risk of an earlier ANM compared to nonsmokers, and women with an active lifestyle had a later ANM (Dratva et al., 2009; Gudmundsdottir, Flanders, & Augestad, 2013; Stepaniak et al., 2013). Emaus et al. (2013) suggested that physical activity was associated with a later ANM only in former- or current-heavy smokers, and had no effect on ANM in the absence of smoking. High parity (Dratva et al., 2009; Kaczmarek, 2007; Martin et al., 2006; Mishra et al., 2017; Morris et al., 2012) and oral contraceptive use (Kaczmarek, 2007; Stepaniak et al., 2013; Zsakai, Mascie-Taylor, & Bodzsar, 2015) have been associated with later ANM. Inconsistent findings have been reported in relation to body mass index (BMI) (Gold, 2011; Tao et al., 2015).

Some studies in women of European descent examined dietary habits (Martin et al., 2006; Nagel, Altenburg, Nieters, Boffetta, & Linseisen, 2005; Stepaniak et al., 2013), showing inconsistent results for vegetables, carbohydrates (Nagel et al., 2005; Stepaniak et al., 2013), and fiber intake (Martin et al., 2006; Nagel et al., 2005). While Nagel et al. (2005) in the Heidelberg sub-cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) showed a later ANM with higher intake of vegetables, carbohydrates and fiber, no effect on menopause was reported from intakes of fats and proteins (Martin et al., 2006). Further, alcohol intake has been associated with a later ANM (Taneri et al., 2016).

Since ANM differs across countries and few studies included Spanish participants (Dratva et al., 2009; Reynolds & Obermeyer, 2005), our aim was to evaluate the role of lifestyle factors (including educational level, BMI, physical activity, and tobacco), recent diet (including alcohol consumption), menstrual factors, and reproductive history in ANM in the Spanish sub-cohort of the EPIC cohort.

2 | MATERIALS AND METHODS

The EPIC-Spain sub-cohort includes 25 808 women, of which 15 844 were pre-menopausal at baseline, aged 29-67 years (68.6% older than 40 years), who were recruited between 1992 and 1996 from five regions of Spain: Asturias, Granada, Murcia, Navarra and San Sebastian. All participants signed an informed consent form, and this study was approved by the Ethics Committee of the Bellvitge Hospital (Barcelona). At recruitment, face-to-face interviews were performed, and validated self-administrated quantitative questionnaires structured by meals, which included an exhaustive list of different types of foods, were used to collect diet information. Further, information on lifestyle and anthropometric measurements was collected. The questionnaire on physical activity consisted of three different types of physical activity assessment: occupational, recreational, and household activity. Since the occupational physical activity data did not include the duration and the frequency of the activity, it was not possible to combine all of the activity collected into one overall variable expressed as the MET-hours/week. Thus, we used physical activity level based on the validated Cambridge Physical Activity Index, where work activity and leisure time physical activity were combined (Supporting Information Table 1) (Wareham et al., 2003). Questionnaires collected information on menstrual factors, reproductive history, and exogenous hormones used (González et al., 2004; Riboli et al., 2002). After a median of 3 years, 15 659 women completed a short follow-up questionnaire, also including information on reproductive history.

ANM was defined as the age at the last menstrual period (years) for those women without menstrual periods for at least 12 consecutive months. Women who reported bilateral oophorectomy or hysterectomy or use of menopausal hormone therapy (natural menopause can be masked by bleeding caused by the therapy [MedlinePlus, 2018]) at baseline or during the follow-up were excluded from the analysis ($n = 1645$). Furthermore, women with possible underlying menopausal symptoms, (oral contraceptive [OC] users older than 40 years, $n = 230$) were also excluded. Thus, 13 784 pre-menopausal women were eligible for the analysis at baseline, and completed the follow-up questionnaire. Nine women were excluded because they did not report their age or menopause status during follow-up.

Women who reported an ANM at follow-up that was younger than the age at recruitment were excluded from the analysis ($n = 680$). Women with ages at recruitment <35 years were considered not at risk of developing menopause during the follow-up, so were excluded from the present analysis ($n = 284$). Finally, since dietary variables were studied, 249 women without dietary information or who had extreme or implausible caloric intake (top or bottom 1% of the ratio of energy intake to estimated energy requirement [Ferrari et al., 2002]) were excluded. A total of 12 562 pre-menopausal women who were at risk of becoming menopausal during follow-up were included in the present analysis.

The following baseline lifestyle variables were included in the analysis: physical activity (including leisure and work

activity: inactive, moderately inactive, moderately active, and active), BMI (kg/m^2), height (cm), educational level (categories were based on the Spanish education system: none, general basic education, job training, high school, and university), smoking status (never, former, current), number of cigarettes for current smokers, and time since quitting smoking for former smokers. Passive smoking information was not collected in the EPIC-Spain sub-cohort.

Dietary variables included intakes of fruit and vegetables, legumes, cereals, fish, dairy products, meat (including red, white, and processed meat), olive oil, and lifetime alcohol intake. Potentially estrogenic foods and dietary components including soy products, polyphenols (isoflavones and lignans, which were estimated using composition databases [Zamora-Ros et al., 2012]), fiber, vitamin D, nuts, and percentage of energy from fat and from carbohydrates, were also evaluated. Further, adherence to the Mediterranean diet was analyzed using the adapted relative Mediterranean diet (arMED) index (Buckland et al., 2013). The arMED score consists of a 16-point scale that incorporates eight key components of the Mediterranean diet (MD). For the six components presumed to fit the MD; fruits (including nuts and seeds), vegetables (excluding potatoes), legumes, fish (fresh or frozen, excluding fish products and preserved fish), olive oil and cereals, a score of 0-2 was assigned to tertiles of intake. The scoring was inverted for the components presumed to not fit MD, meat and dairy products. The points were summed to define the arMED score that ranges from 0 (no adherence) to 16 (maximum adherence) and represents the level of adherence to the MD. Alcohol was not included in the arMED index since it is a potential risk factor for a later ANM. Information on past alcohol intake was assessed as average number of glasses of beverage consumed per week at ages 20, 30, 40, and 50 years. Weighted average intake with weights equal to the amount of time of exposure to alcohol during decades was calculated to determine average lifetime alcohol intake (Klipstein-Grobusch et al., 2002).

Variables for menstrual factors were age at menarche (<12, 12, 13, 14, >14 years), mean time between menarche and regular menses (regular since menarche, regular after 1 year, regular after ≥2 years, and irregular the 1st 10 years/irregular since 1st pregnancy). Reproductive history included number of pregnancies (including live and stillbirth, and induced and spontaneous abortion; 0, 1, 2, 3, ≥4 pregnancies), number of full-term pregnancies (including live and stillbirth; 0, 1, 2, 3, ≥4 births), age at first full-term pregnancy (<20, 20-23, 24-27, >27 years), breastfeeding (yes, no). OC variables were: use (yes, no), duration (based on tertiles; 1, 2-4, >4 years), and age at first use (<25, 25-30, ≥31 years). Further analysis was performed to evaluate differences between high and low doses of OC (where low dose was considered if OC was prescribed after the year 1972 [≤50 μg ethinyl estradiol] [de Vries et al., 2001]). Finally, unilateral oophorectomy (yes, no) was analyzed.

Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% confidence interval (CIs) for each exposure variable and incident menopause. Age at recruitment was used as entry time, and ANM (the event) or age at the end of follow-up, were used as exit time. All models were stratified by center and age at recruitment. To account for all sources of hormone exposure and hormonal modifying factors, mutually adjusted models were evaluated for all identified factors. Restricted cubic splines with 3-5 knots were used to explore the shape of the dose-response for each continuous exposure variable. Effect-measure modification by smoking status (never, former, current) was evaluated using a likelihood ratio test (LRT) in the mutually adjusted model.

All analyses were performed using SAS v. 9.4 (Cary, North Carolina, USA).

3 | RESULTS

After a median of 3 years of follow-up, 1166 premenopausal women at baseline (9.3%) from EPIC-Spain experienced natural menopause with a median age of 51 years [percentile 25-75, 49-53 years], approximately 5% of whom were under 45 years and 3% were older than 55 years. Baseline characteristics of the participants according to ANM quartiles are reported in Table 1. Women with youngest ANM (≤48 years) were mostly overweight, inactive, and had basic education. Further, they had the highest proportion of ever smokers, and OC users who started between 25 and 30 years, and with OC duration shorter than 1 year.

Compared with never-smoking women, current smokers had increased risk of having an earlier ANM (HR: 1.26; 95% CI 1.05-1.51); however, an inverse association was observed in former smokers (HR: 0.77; 95%CI 0.60-0.99) (Table 1). Women whose menses were irregular had a later ANM compared with women with regular menses (HR: 0.70; 95%CI 0.55-0.89) (Table 1). Increasing number of pregnancies was associated with a later ANM ($HR_{\geq 4 vs never}$: 0.70; 95%CI 0.56-0.88). Among ever-pregnant women, a similar, but non-significant, pattern was seen for number of full-term pregnancies and a later ANM. A statistically significant inverse association (later ANM) was observed in women who reported ever use of OC (HR: 0.85; 95%CI 0.75-0.97) (Table 1). Among OC users, women who started treatment between the ages of 25 and 30 years compared with those who started after the age of 31 years of age had a reduced risk of an earlier ANM (HR: 0.69; 95%CI 0.55-0.87). Physical activity was not associated with ANM in this study. Null associations were observed for the rest of the studied variables, including dietary habits, lifetime alcohol intake, and the Mediterranean dietary index (Table 1). Alcohol intakes at 20 years, at 30 years, and 40 years were also analyzed and no associations were observed (data not shown). Cubic

TABLE 1 Descriptive characteristics by age at natural menopause (ANM), and hazard ratios for ANM and baseline lifestyle, dietary, and reproductive factors in EPIC-Spain women

	Post-menopausal[a] (n = 1166, 9.3%)	Age at natural menopause[a]		
		Q1 (≤48-year, n = 268)	Q4 (≥53-year, n = 291)	HR (95%CI) for ANM[b]
Age at recruitment (years)	49.4 (47.4-51.3)	45.72 (44.37-46.69)	52.81 (52.00-53.76)	
LIFESTYLE				
BMI (kg/m^2)				
<25: normal[c]	258 (22.1)	77 (28.7)	38 (13.1)	1.00 (referent)
25-30: Overweight	496 (42.5)	113 (42.2)	120 (41.2)	0.89 (0.76-1.04)
>30: Obese	412 (35.3)	78 (29.1)	133 (45.7)	0.89 (0.75-1.04)
P-trend				0.195
Height (cm)				
Q1 (≤153.0)	291 (25.0)	69 (25.8)	84 (28.9)	1.00 (referent)
Q2 (>153.0-156.3)	262 (22.5)	67 (25.0)	70 (24.1)	1.13 (0.94-1.32)
Q3 (>156.3-159.0)	247 (21.2)	56 (20.9)	54 (18.6)	1.06 (0.90-1.27)
Q4 (>159.0-162.5)	206 (13.7)	42 (15.7)	48 (16.5)	0.99 (0.82-1.19)
Q5 (>162.5)	160 (13.7)	34 (12.7)	35 (12.0)	0.93 (0.76-1.13)
P-trend				0.399
Physical activity index				
Inactive	609 (52.2)	136 (50.8)	173 (59.5)	1.00 (referent)
Moderately inactive	408 (35)	87 (32.5)	95 (32.7)	1.02 (0.90-1.16)
Moderately active	119 (10.2)	33 (12.3)	20 (6.9)	1.06 (0.87-1.30)
Active	30 (2.6)	12 (4.5)	3 (1.0)	0.80 (0.55-1.17)
P-trend				0.846
Educational level				
None	506 (43.4)	96 (35.8)	152 (52.2)	1.00 (referent)
General basic education	500 (42.9)	128 (47.8)	107 (36.8)	0.95 (0.83-1.08)
Job training	42 (3.6)	14 (5.2)	6 (2.1)	0.85 (0.61-1.17)
High school	39 (3.3)	9 (3.4)	8 (2.8)	0.94 (0.67-1.31)
University	70 (6)	20 (7.5)	14 (4.8)	0.85 (0.66-1.09)
Missing	9 (0.8)	1 (0.4)	4 (1.4)	
P-trend				0.154
Smoking status				
Never	943 (80.9)	180 (67.2)	265 (91.1)	1.00 (referent)
Former	68 (5.8)	21 (7.8)	9 (3.1)	0.77 (0.60-0.99)
Current	154 (13.2)	67 (25.0)	16 (5.5)	1.26 (1.05-1.51)
Unknown	1 (0.1)		1 (0.3)	
Smoking status and intensity of smoking				
Never	943 (80.9)	180 (67.2)	265 (91.1)	1.00 (referent)
Former, quit 20+ years	11 (0.9)	2 (0.8)	3 (1.0)	0.57 (0.32-1.04)
Former, quit 11-20 years	16 (1.4)	3 (1.1)	4 (1.4)	0.66 (0.40-1.09)
Former, quit ≤ 10 years	41 (3.5)	16 (6.0)	2 (0.7)	0.97 (0.71-1.34)
Current, 1-15 cig/day	102 (8.7)	39 (14.6)	10 (3.4)	1.27 (1.03-1.57)
Current, 15+ cig/day	48 (4.1)	27 (10.1)	4 (1.4)	1.31 (0.97-1.76)
Current, pipe/cigar/occasional cigarette smokers	2 (0.2)	1 (0.4)	1 (0.3)	1.28 (0.31-5.23)
Current/former, missing	2 (0.2)		1 (0.3)	0.36 (0.09-1.50)
Unknown	1 (0.1)		1 (0.3)	
P-trend[d]				0.036
DIET[e]				
Vegetables (100 g/2000 kcal/day)	2.5 (1.6-3.4)	2.53 (1.56-3.38)	2.44 (1.49-3.25)	1.00 (0.96-1.04)
Fruits (100 g/2000 kcal/day)	3.2 (2.0-4.8)	2.93 (1.76-4.75)	3.41 (2.15-4.94)	0.98 (0.96-1.01)
Legumes (50 g/2000 kcal/day)	0.8 (0.5-1.2)	0.80 (0.47-1.14)	0.83 (0.48-1.23)	1.04 (0.94-1.15)

(*Continues*)

TABLE 1 (Continued)

	Post-menopausal[a] (n = 1166, 9.3%)	Age at natural menopause[a]		
		Q1 (≤48-year, n = 268)	Q4 (≥53-year, n = 291)	HR (95%CI) for ANM[b]
Cereals (50 g/2000 kcal/day)	3.4 (2.5-4.5)	3.42 (2.45-4.53)	3.54 (2.67-4.53)	0.99 (0.95-1.03)
Fish (50 g/2000 kcal/day)	1.0 (0.6-1.6)	0.96 (0.55-1.45)	0.93 (0.56-1.52)	1.06 (0.99-1.14)
Dairy products (100 g/2000 kcal/day)	3.2 (2.1-4.5)	3.01 (1.98-4.18)	3.22 (2.21-4.78)	0.97 (0.94-1.00)
Meat (100 g/2000 kcal/day)	1.1 (0.8-1.5)	1.12 (0.90-1.44)	1.15 (0.82-1.50)	1.02 (0.90-1.15)
Olive oil (10 g/2000 kcal/day)	2.2(1.3-3.0)	2.18 (1.14-3.13)	2.07 (1.30-3.04)	1.04 (0.99-1.09)
arMED score[f]				
Low (0-5)	187 (16.0)	43 (16.0)	46 (15.8)	1.00 (referent)
Medium (6-9)	621 (53.3)	137 (51.1)	157 (54.0)	0.97 (0.82-1.15)
High (10-16)	358 (30.7)	88 (32.8)	88 (30.2)	0.98 (0.82-1.18)
P-trend				0.982
Lifetime alcohol intake (g/day)				
Never-consumer	441 (37.8)	102 (38.1)	112 (38.5)	1.00 (referent)
≤6	432 (37.0)	102 (38.1)	105 (36.1)	0.93 (0.81-1.07)
>6-12	129 (11.1)	23 (8.6)	32 (11.0)	0.89 (0.73-1.10)
>12	159 (13.6)	41 (15.3)	39 (13.4)	1.09 (0.90-1.33)
Missing	5 (0.4)		3 (1.0)	
P-trend				0.748
Isoflavones (mg/day)	0 (0-0.1)	0.04 (0.02-0.06)	0.04 (0.03-0.07)	0.91 (0.69-1.20)
Lignans (mg/day)	1.5 (1.1-2)	1.48 (1.08-1.90)	1.52 (1.13-2.02)	1.01 (0.90-1.13)
Vitamin D (μg/day)	3.1 (1.9-4.5)	3.03 (1.84-4.32)	3.14 (1.78-4.50)	1.01 (0.98-1.04)
Fiber (g/day)	21.4 (17.5-26.4)	21.17 (17.07-25.19)	21.84 (17.96-27.23)	1.00 (0.99-1.01)
Nuts (g/day)				
Nonconsumers	723 (62.0)	169 (63.1)	182 (62.5)	1.00 (referent)
≤5	198 (17.0)	43 (16.0)	45 (15.5)	1.01 (0.83-1.22)
>5	245 (21.0)	56 (20.9)	64 (22.0)	1.01 (0.85-1.19)
P-trend				0.910
% of energy from fat	12.8 (9.6-16.4)	12.8 (9.6-16.5)	12.6 (9.4-16.7)	1.01 (0.91-1.13)
% of energy from carbohydrates	41.9 (37.7-46.1)	41.9 (38.4-45.9)	42.2 (37.5-46.9)	0.99 (0.99-1.00)
REPRODUCTIVE HISTORY				
Age at menarche (years)				
<12	219 (18.8)	50 (18.7)	44 (15.1)	1.00 (referent)
12	231 (19.8)	70 (26.1)	44 (15.1)	1.05 (0.87-1.27)
13	289 (24.8)	57 (21.3)	73 (25.1)	1.00 (0.84-1.20)
14	238 (20.4)	56 (20.9)	64 (22.0)	0.85 (0.70-1.02)
>14	188 (16.1)	35 (13.1)	65 (22.3)	1.02 (0.83-1.24)
Missing	1 (0.1)		1 (0.3)	
P-trend				0.289
Mean time between menarche and regular menses				
Regular since menarche	1010 (86.6)	232 (86.6)	250 (85.9)	1.00 (referent)
Regular after 1 year	45 (3.9)	9 (3.4)	10 (3.4)	0.95 (0.70-1.29)
Regular after ≥2 years	27 (2.3)	8 (3.0)	6 (2.1)	0.85 (0.57-1.25)
Irregular the 1st 10 years/irregular since 1st pregnancy	75 (6.4)	17 (6.3)	24 (8.3)	0.70 (0.55-0.89)
Missing	9 (0.8)	2 (0.8)	1 (0.3)	
P-trend				0.003
Number of pregnancies				
0	106 (9.1)	25 (9.3)	20 (6.9)	1.00 (referent)
1	75 (6.4)	23 (8.6)	17 (5.8)	0.88 (0.65-1.20)
2	298 (25.6)	82 (30.6)	71 (24.4)	0.73 (0.58-0.91)

(*Continues*)

TABLE 1 (Continued)

	Post-menopausal[a] (n = 1166, 9.3%)	Age at natural menopause[a]		
		Q1 (≤48-year, n = 268)	Q4 (≥53-year, n = 291)	HR (95%CI) for ANM[b]
3	337 (28.9)	75 (28.0)	79 (27.2)	0.78 (0.62-0.98)
≥4	344 (29.5)	62 (23.1)	103 (35.4)	0.70 (0.56-0.88)
Missing	6 (0.5)	1 (0.4)	1 (0.3)	
P-trend				0.006
Number of full-term pregnancies				
0	9 (0.8)	1 (0.4)	2 (0.7)	1.12 (0.55-2.25)
1	85 (7.3)	28 (10.5)	19 (6.5)	1.00 (referent)
2	392 (33.6)	110 (41.0)	85 (29.2)	0.90 (0.71-1.15)
3	340 (29.2)	68 (25.4)	89 (30.6)	0.86 (0.67-1.11)
≥4	228 (19.6)	35 (13.1)	75 (25.8)	0.81 (0.62-1.06)
Missing	6 (0.5)	1 (0.4)	1 (0.3)	
P-trend				0.077
Age at first full-term pregnancy (years)[g]				
<20	46 (3.9)	9 (3.7)	10 (3.7)	1.00 (referent)
20-23	338 (29.0)	103 (42.6)	77 (28.6)	0.99 (0.73-1.36)
24-27	481 (41.3)	98 (40.5)	141 (52.4)	1.03 (0.75-1.40)
>27	180 (15.4)	31 (12.8)	40 (14.9)	1.01 (0.72-1.41)
Missing	6 (0.5)	1 (0.4)	1 (0.4)	
P-trend				0.773
Breastfeeding[g]				
No	140 (13.3)	47 (19.5)	21 (7.8)	1.00 (referent)
Yes	912 (86.5)	194 (80.5)	242 (92.2)	0.91 (0.76-1.09)
Missing	8 (0.2)			
OC use				
No	773 (66.3)	152 (56.7)	219 (75.3)	1.00 (referent)
Yes				0.85 (0.75-0.97)
Age at start OC use (years)				
<25	52 (4.5)	31 (26.7)	3 (4.2)	0.73 (0.52-1.03)
25-30	165 (14.2)	52 (44.8)	26 (36.1)	0.69 (0.55-0.87)
≥31	174 (14.9)	33 (28.4)	41 (56.9)	1.00 (referent)
Missing	2 (0.2)		2 (2.8)	
P-trend				0.009
Duration OC use (years)				
≤1	173 (44.0)	49 (42.2)	28 (38.9)	1.00 (referent)
2-4	99 (25.2)	26 (22.4)	19 (26.4)	0.81 (0.63-1.04)
>4	119 (30.3)	41 (35.3)	23 (31.9)	1.10 (0.86-1.41)
Missing	2 (0.5)		2 (2.8)	
P-trend				0.590
Unilateral oophorectomy				
No	1151 (98.7)	265 (98.9)	287 (98.6)	1.00 (referent)
Yes	15 (1.3)	3 (1.1)	4 (1.4)	1.04 (0.63-1.75)

[a] n(%) for qualitative variables and median (25th-75th percentiles) for quantitative variables.
[b] HR > 1 indicates that the risk factor was associated with an earlier ANM and a HR <1 indicates that the risk factor was associated with a later ANM. Crude cox models stratified by center and age at recruitment.
[c] Including underweight (21 pre-menopausal/1 post-menopausal).
[d] *P*-trend excluding the categories "current, pipe/cigar/occasional cigarette smokers," "current/former, missing," and "Unknown."
[e] Adjusted for total energy intake.
[f] Adjusted for total energy intake and lifetime alcohol intake.
[g] In parous women.

TABLE 2 Mutually adjusted cox model for smoking status, time since regular menses, oral contraceptive use, and age at natural menopause in EPIC-Spain women

	Post-menopausal	HR (95%CI)[a]	*P*-trend
Smoking status			
Never	934	1.00 (referent)	
Former	67	0.78 (0.61-1.01)	
Current	151	1.29 (1.08-1.55)	
Mean time between menarche and regular menses			
Regular since menarche	1002	1.00 (referent)	0.004
Regular after 1 year	45	0.95 (0.70-1.30)	
Regular after ≥ 2 years	27	0.86 (0.58-1.26)	
Irregular the 1st 10 years/irregular since 1st pregnancy	74	0.70 (0.55-0.90)	
OC use and age at start (years)			
Never use	760	0.94 (0.79-1.11)	0.009
<25	52	0.82 (0.59-1.13)	
25-30	163	0.72 (0.58-0.89)	
≥31	173	1.00 (referent)	
Number of pregnancies			
0	104	1.00 (referent)	0.027
1	74	0.91 (0.67-1.24)	
2	297	0.76 (0.61-0.97)	
3	333	0.83 (0.65-1.04)	
≥4	340	0.74 (0.56-0.94)	

OC, Oral contraceptive.
[a] HR > 1 indicates that the risk factor was associated with an earlier ANM and a HR <1 indicates that the risk factor was associated with a later ANM. Mutually adjusted Cox model stratified by center and age at recruitment.

spline modeling demonstrated that all continuous variables were linear in relation to ANM.

A mutually adjusted Cox model was evaluated including the variables that were statistically significant in the univariate analysis: smoking status, number of pregnancies, time since regular menses, OC use, and age at first use of OC. OC use and age at starting OC use were combined into one variable (never use, <25, 25-30, ≥31 years), to obtain comparable results with those observed in Table 1 (the referent category in the model was ≥31 years). Table 2 gives the final multivariate Cox model where the risk of an earlier ANM in current *vs* never smokers was 1.29 (95%CI 1.08-1.55), while the risk of a later ANM in former *vs* never smokers was 0.78 (95%CI 0.61-1.01). For women who started taking OC pills between ages from 25 to 30 years, compared with the age of at least 31 years, relative risk was 0.72(95%CI 0.58-0.89), while women with irregular menses had a later ANM ($HR_{irregular vs regular}$: 0.70; 95%CI 0.55-0.90). Finally, a greater number of pregnancies (≥4) decreased the risk of an earlier ANM compared with women who never became pregnant (HR: 0.74; 95%CI 0.56-0.94) (Table 2). There was no evidence for effect-measure modification of ANM relations by smoking status in the mutually adjusted model (all *P*-values >0.1) (data not shown).

A sensitivity analysis was performed to qualitatively evaluate estrogen dose changes for OC preparations (Supporting Information Table 2). Short-term (≤4 years) use of high dose OCs (prescription year ≤1972) was associated with a reduction of the risk of an earlier ANM compared to never never-use of OC (HR: 0.74; 95%CI 0.58-0.93).

4 | DISCUSSION

The median ANM in our study population of 12 562 premenopausal Spanish women was 51 years. Current smokers had a 29% increased risk of experiencing menopause at younger ages compared with never smokers. A later ANM was observed in women who started using OC between the ages of 25 and 30 years. Finally, a later ANM was associated with a higher number of pregnancies and with reporting a history of irregular menses. No associations were observed for dietary variables and the remaining lifestyle and reproductive history factors.

In previous studies, elevated intakes of vegetables were inconsistently related with ANM (Nagel et al., 2005; Stepaniak et al., 2013). Stepaniak et al. (2013) suggested that higher intakes of vegetables were associated with an earlier ANM, while Nagel et al. (2005) observed a later ANM. Our results showed no association between vegetable intake, high adherence to arMED (characterized by elevated intakes of fruit and vegetables), and ANM. In contrast with previous studies, our results were based on a shorter follow-up and a lower cumulative incidence of menopause (EPIC-Spain: 9.3%, Nagel: 21%, and Stepaniak: 72%). Further, we only evaluated dietary habits a median of 3 years after the age of

40 years (ie, we did not assess diet during earlier periods of the fertile lifespan).

Alcohol influences serum concentrations of androgen and estrone (E_1) by increasing their levels in blood (Rinaldi et al., 2006), and some authors have reported that alcohol may be inversely associated with ANM (Taneri et al., 2016). No association between alcohol intake and ANM was observed in the present study.

The association between current smoking and an earlier ANM is well-established (Sun et al., 2012). Cigarette smoking has an anti-estrogenic effect in women and is considered an important sex hormone modifier (Kapoor & Jones, 2005). In our mutually adjusted model we observed a 29% increased risk of having an earlier ANM in women who reported that they smoked at baseline, and an unexpected nonsignificant risk reduction in former smokers. Hayatbakhsh, Clavarino, Williams, Sina, and Najman (2012) suggested that the effect of smoking on ANM is reversible if women quit during earlier reproductive life (Hayatbakhsh et al., 2012). Although the inverse association in former smokers was not statistically significant, additional studies are needed before any conclusions can be made.

A literature review observed inconsistent findings in relation to BMI and ANM, and the author suggested that this was due to differences in the study design and adjustment variables (Gold, 2011). A meta-analysis by Tao et al. (2015) observed that underweight women were younger at the time of menopause, compared with normal-weight women. Further, they observed that overweight women underwent later menopause possibly due to higher estrogen levels in adipose tissue (Tao et al., 2015). We observed associations between a later ANM and overweight and obesity, but neither was statistically significant. In the Spanish-EPIC cohort most of the women (n = 8018, 63.8%) were classified as obese or overweight, 36% as normal weight, and only 22 (0.2%) as underweight.

Consistent results in relation to parity and OC use and a later ANM have been reported (Dratva et al., 2009; Kaczmarek, 2007; Martin et al., 2006; Morris et al., 2012; Stepaniak et al., 2013; Zsakai et al., 2015). We observed that women with more than one pregnancy (including full-term pregnancies and abortions) and OC users were older at the moment of menopause, especially women who started OC use between the ages of 25 and 30 years compared with those who started at the age of 31 or later. The mechanism by which OC use might affect the reproductive lifespan is not yet clear. Some authors suggested that a later ANM among OC users could be caused by a delay in the depletion of oocytes by OCs (Gold, 2011). Use of OC alters hormone levels, and this further depends on the type, dosage, and mode of administration of OC. It has been observed by others that FSH, LH, and estradiol serum concentration levels decrease during OC use (D'Arpe et al., 2016). Inconsistent results were reported in relation to anti-Mullerian hormone (AMH) which is used as an ovarian aging marker and an indicator of a woman's ovarian reserve. While some studies observed that OC use did not influence AMH concentrations, other studies reported lower levels of AMH in OC users than in non-users (D'Arpe et al., 2016).

OC formulations have changed since they were introduced in the 1960s. Early OC preparations contained >50 μg ethinyl estradiol, and doses decreased in the 1970s to 30 μg ethinyl estradiol. Current doses could be as low as 15 μg. The "Doorlopend Onderzoek Morbiditeit/Mortaliteit" (DOM-3) cohort compared the effect of long-term use of high and low doses of OC with non-OC users in relation to the ANM. They observed that DOM-3 participants with long-term (≥3 years) use of high doses of OC (prescribed prior to 1972) had an increased risk of earlier menopause compared to non-users (HR:1.12; 95%CI 1.03-1.21) (de Vries et al., 2001), and there was no effect of lower doses of OC, possibly due to moderate exposure to estrogens and progestin. In Spain, the use of OC as a contraceptive method was legalized in 1978; previously it was prescribed to regulate menstrual cycles. In the EPIC-Spain sub-cohort, 51.5% of OC-users started the treatment before 1978 and only 13% before 1972. Our results by dose of OC are not in agreement with the DOM-3 study, but are in accordance with the hypothesis that a reduction in FHS and LH levels may delay ANM. We observed that short-term users of high dose OCs had a reduced risk of earlier menopause compared to non-users. In EPIC-Spain, long-term users of high dose OCs reported last use of OC after the year 1972, so it is possible that during the period that they were taking OC, the formulation changed from high to low dose and any possible effect on ANM was diminished. These results should be interpreted with caution because information on actual doses was not collected in our study, and the cut-off we used was somewhat arbitrary.

Higher physical activity levels have been associated with a later ANM (Dratva et al., 2009; Emaus et al., 2013; Gudmundsdottir et al., 2013; Stepaniak et al., 2013). Our data suggested that ANM is not associated with physical activity at baseline (after the age of 40 years). Physical activity tends to reduce circulating sex hormones, especially total and free circulating estradiol concentrations (Ennour-Idrissi, Maunsell, & Diorio, 2015) causing irregular menstrual cycles or amenorrhea in extremely active women. Our results suggest that irregular menses were associated with a later ANM, in agreement with an EPIC-Heidelberg sub-cohort study where women with irregular menses during the first 5 years after menarche had a later ANM compared to women who had regular menses since the first year of menarche (HR: 0.67; 95%CI 0.5-0.89) (Nagel et al., 2005). In our study, the observed association remained significant after adjusting for smoking status and OC use. Polycystic ovary syndrome (PCOS) is associated with irregular menses; and almost 10% of women worldwide are affected by PCOS. A recent study found that women with PCOS had longer reproductive life

spans. Women diagnosed with PCOS had higher testosterone levels, and a higher number of follicles (Li, Eriksson, Czene, Hall, & Rodriguez-Wallberg, 2016). Measures of FSH and LH levels in serum provide an accurate diagnosis of menopause status (NAMS, 2017). Unfortunately, information on FSH and LH levels was not available in the present study.

To our knowledge this is the largest prospective study of lifestyle, diet, menstrual factors and reproductive history, and age at natural menopause carried out in five Spanish regions. Further, to the best of our knowledge, this is the first study to examine the Mediterranean dietary pattern (using the arMED index) and ANM.

Our results support previous evidence that smokers undergo earlier menopause; however, women who took OC, had a higher number of pregnancies, or had irregular menses had a later ANM. Our results suggest that dietary habits assessed after the age of 40 years have no effect on ANM. Further investigations of the role of modifiable factors (including also dietary habits in earlier life, second-hand tobacco smoke exposure, and exposure to environmental contaminants) on ANM using FSH and LH levels in serum to more accurately diagnose menopause status are needed.

ACKNOWLEDGEMENT

This work was supported by the Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia, Navarra, and the Catalan Institute of Oncology.

AUTHOR CONTRIBUTION

LLB, KG, MOS, and EJD analyzed and interpreted the data. LLB and EJD wrote the manuscript. LLB designed the study. MDC, MJS, NL, AB, JRQ, ESF, SCY, BGP, and AA collected the data and provided critical comments on the manuscript.

ORCID

Leila Lujan-Barroso http://orcid.org/0000-0001-6224-1764

REFERENCES

Buckland, G., Travier, N., Cottet, V., González, C. A., Luján-Barroso, L., Agudo, A., … Riboli, E. (2013). Adherence to the mediterranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. *International Journal of Cancer*, *132*, 2918–2927.

Daan, N. M. P., & Fauser, B. C. J. M. (2015). Menopause prediction and potential implications. *Maturitas*, *82*, 257–265.

D'Arpe, S., Di Feliciantonio, M., Candelieri, M., Franceschetti, S., Piccioni, M. G., & Bastianelli, C. (2016). Ovarian function during hormonal contraception assessed by endocrine and sonographic markers: A systematic review. *Reproductive Biomedicine Online*, *33*, 436–448.

de Vries, E., den Tonkelaar, I., van Noord, P. A., van der Schouw, Y. T., te Velde, E. R., & Peeters, P. H. (2001). Oral contraceptive use in relation to age at menopause in the DOM cohort. *Human Reproduction (Oxford, England)*, *16*, 1657–1662.

Dratva, J., Gómez Real, F., Schindler, C., Ackermann-Liebrich, U., Gerbase, M. W., Probst-Hensch, N. M., … Zemp, E. (2009). Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. *Menopause (New York, N.Y.)*, *16*, 385–394.

Emaus, A., Dieli-Conwright, C., Xu, X., Lacey, J. V., Ingles, S. A., Reynolds, P., … Henderson, K. D. (2013). Increased long-term recreational physical activity is associated with older age at natural menopause among heavy smokers: The California teachers study. *Menopause (New York, N.Y.)*, *20*, 282–290.

Ennour-Idrissi, K., Maunsell, E., & Diorio, C. (2015). Effect of physical activity on sex hormones in women: A systematic review and meta-analysis of randomized controlled trials. *Breast Cancer Research: BCR*, *17*, 139.

Ferrari, P., Slimani, N., Ciampi, A., Trichopoulou, A., Naska, A., Lauria, C., … Riboli, E. (2002). Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European prospective investigation into cancer and nutrition (EPIC). *Public Health Nutrition*, *5*, 1329–1345.

Gold, E. B. (2011). The timing of the age at which natural menopause occurs. *Obstetrics and Gynecology Clinics of North America*, *38*, 425–440.

González, C. A., Navarro, C., Martínez, C., Quirós, J. R., Dorronsoro, M., Barricarte, A., … Berenguer, A. (2004). The European prospective investigation about cancer and nutrition (EPIC). *Revista Española de Salud Pública*, *78*, 167–176.

Gudmundsdottir, S. L., Flanders, W. D., & Augestad, L. B. (2013). Physical activity and age at menopause: The Nord-Trøndelag population-based health study. *Climacteric: The Journal of the International Menopause Society*, *16*, 78–87.

Hayatbakhsh, M. R., Clavarino, A., Williams, G. M., Sina, M., & Najman, J. M. (2012). Cigarette smoking and age of menopause: A large prospective study. *Maturitas*, *72*, 346–352.

Kaczmarek, M. (2007). The timing of natural menopause in Poland and associated factors. *Maturitas*, *57*, 139–153.

Kapoor, D., & Jones, T. H. (2005). Smoking and hormones in health and endocrine disorders. *European Journal of Endocrinology / European Federation of Endocrine Societies*, *152*, 491–499.

Klipstein-Grobusch, K., Slimani, N., Krogh, V., Keil, U., Boeing, H., Overvad, K., … Riboli, E. (2002). Trends in self-reported past alcoholic beverage consumption and ethanol intake from 1950 to 1995 observed in eight European countries participating in the European investigation into cancer and nutrition (EPIC). *Public Health Nutrition*, *5*, 1297–1310.

Li, J., Eriksson, M., Czene, K., Hall, P., & Rodriguez-Wallberg, K. A. (2016). Common diseases as determinants of menopausal age. *Human Reproduction (Oxford, England)*. *31*, 2856-2864 doi:https://doi.org/10.1093/humrep/dew264

Martin, L. J., Greenberg, C. V., Kriukov, V., Minkin, S., Jenkins, D. J. A., & Boyd, N. F. (2006). Intervention with a low-fat, high-carbohydrate diet does not influence the timing of menopause. *The American Journal of Clinical Nutrition*, *84*, 920–928.

MedlinePlus (2018) Types of hormone therapy: MedlinePlus Medical Encyclopedia. Retrieved March 29, 2018, from https://medlineplus.gov/ency/patientinstructions/000695.htm

Mishra, G. D., Pandeya, N., Dobson, A. J., Chung, H.-F., Anderson, D., Kuh, D., … Weiderpass, E. (2017). Early menarche, nulliparity and the risk for premature and early natural menopause. *Human Reproduction (Oxford, England)*. *32*, 679-686 doi:https://doi.org/10.1093/humrep/dew350

Morris, D. H., Jones, M. E., Schoemaker, M. J., McFadden, E., Ashworth, A., & Swerdlow, A. J. (2012). Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: Analyses from the breakthrough generations study. *American Journal of Epidemiology*, *175*, 998–1005.

Nagel, G., Altenburg, H.-P., Nieters, A., Boffetta, P., & Linseisen, J. (2005). Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*, *52*, 337–347.

NAMS (The North American Menopasue Society) (2017) Menopause Glossary, Menopause Resources. Retrieved February 15, 2017, from https://www.menopause.org/for-women/menopause-glossary

Reynolds, R. F., & Obermeyer, C. M. (2005). Age at natural menopause in Spain and the United States: Results from the DAMES project. *American Journal of Human Biology*, *17*, 331–340.

Riboli, E., Hunt, K. J., Slimani, N., Ferrari, P., Norat, T., Fahey, M., … Saracci, R. (2002). European prospective investigation into cancer and nutrition (EPIC): Study populations and data collection. *Public Health Nutrition*, *5*, 1113–1124.

Rinaldi, S., Peeters, P. H. M., Bezemer, I. D., Dossus, L., Biessy, C., Sacerdote, C., … Kaaks, R. (2006). Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: The European prospective investigation into cancer and nutrition. *Cancer Causes & Control*, *17*, 1033–1043.

Stepaniak, U., Szafraniex, K., Kubinova, R., Malyutina, S., Peasey, A., Pikhart, H., … Bobak, M. (2013). Age at natural menopause in three central and eastern European urban populations: The HAPIEE study. *Maturitas*, *75*, 87–93.

Sun, L., Tan, L., Yang, F., Luo, Y., Li, X., Deng, H.-W., & Dvornyk, V. (2012). Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. *Menopause (New York, N.Y.)*, *19*, 126–132.

Taneri, P. E., Kiefte-de Jong, J. C., Bramer, W. M., Daan, N. M. P., Franco, O. H., & Muka, T. (2016). Association of alcohol consumption with the onset of natural menopause: A systematic review and meta-analysis. *Human Reproduction Update*, *22*, 516–528.

Tao, X., Jiang, A., Yin, L., Li, Y., Tao, F., & Hu, H. (2015). Body mass index and age at natural menopause: A meta-analysis. *Menopause (New York, N.Y.)*, *22*, 469–474.

Wareham, N. J., Jakes, R. W., Rennie, K. L., Schuit, J., Mitchell, J., Hennings, S., & Day, N. E. (2003). Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European prospective investigation into cancer and nutrition (EPIC) study. *Public Health Nutrition*, *6*, 407–413.

Zamora-Ros, R., Knaze, V., Luján-Barroso, L., Kuhnle, G. G. C., Mulligan, A. A., Touillaud, M., … González, C. A. (2012). Dietary intakes and food sources of phytoestrogens in the European prospective investigation into cancer and nutrition (EPIC) 24-hour dietary recall cohort. *European Journal of Clinical Nutrition*, *66*, 932–941.

Zsakai, A., Mascie-Taylor, N., & Bodzsar, E. B. (2015). Relationship between some indicators of reproductive history, body fatness and the menopausal transition in Hungarian women. *Journal of Physiological Anthropology*, *34*, 35.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lujan-Barroso L, Gibert K, Obón-Santacana M, et al. The influence of lifestyle, diet, and reproductive history on age at natural menopause in Spain: Analysis from the EPIC-Spain subcohort. *Am J Hum Biol*. 2018;e23181. https://doi.org/10.1002/ajhb.23181

PAPER – II

Menstrual and Reproductive factors, hormone use, and risk of pancreatic cancer case-control consortium (PanC4).

Lujan-Barroso L, Zhang W, Gao Y-T, Yu H, Bracci PM, Baghurst PA, Bueno-de-Mesquita HB, Gallinger S, Holcatova I, Ji B-T, La Vecchia C, Lagiou P, Li D, Miller AB, Olson S, Scélo G, Serraino D, Zatonski W, Risch HA, Duell E.

Pancreas (2016); 45 (10): 1401-10

Menstrual and Reproductive Factors, Hormone Use, and Risk of Pancreatic Cancer

Analysis From the International Pancreatic Cancer Case–Control Consortium (PanC4)

Leila Lujan-Barroso, MsC, Wei Zhang, PhD,† Sara H. Olson, PhD,‡ Yu-Tang Gao, PhD,† Herbert Yu, PhD,§ Peter A. Baghurst, PhD,|| Paige M. Bracci, PhD,¶ H. Bas Bueno-de-Mesquita, MD, PhD,#**†‡‡ Lenka Foretová, PhD,§§ Steven Gallinger, MD,|||| Ivana Holcatova, MD, PhD,¶¶ Vladimír Janout, MD,## Bu-Tian Ji, MD, PhD,*** Robert C. Kurtz, MD,††† Carlo La Vecchia, MD,‡‡‡ Pagona Lagiou, PhD, MD,§§§|||||| Donghui Li, PhD,¶¶¶ Anthony B. Miller, MD,### Diego Serraino, PhD,**** Witold Zatonski, MD,†††† Harvey A. Risch, PhD, MD,‡‡‡‡ and Eric J. Duell, MS, PhD**

Objectives: We aimed to evaluate the relation between menstrual and reproductive factors, exogenous hormones, and risk of pancreatic cancer (PC).

Methods: Eleven case–control studies within the International Pancreatic Cancer Case–control Consortium took part in the present study, including in total 2838 case and 4748 control women. Pooled estimates of odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using a 2-step logistic regression model and adjusting for relevant covariates.

Results: An inverse OR was observed in women who reported having had hysterectomy ($OR_{yes vs. no}$, 0.78; 95% CI, 0.67–0.91), remaining significant in postmenopausal women and never-smoking women, adjusted for potential PC confounders. A mutually adjusted model with the joint effect for hormone replacement therapy (HRT) and hysterectomy showed significant inverse associations with PC in women who reported having had hysterectomy with HRT use (OR, 0.64; 95% CI, 0.48–0.84).

Conclusions: Our large pooled analysis suggests that women who have had a hysterectomy may have reduced risk of PC. However, we cannot rule out that the reduced risk could be due to factors or indications for having had a hysterectomy. Further investigation of risk according to HRT use and reason for hysterectomy may be necessary.

Key Words: pancreatic cancer, menstrual and reproductive factors, exogenous hormones, hysterectomy, consortium

(*Pancreas* 2016;45: 1401–1410)

Pancreatic cancer (PC) is the 12th most common cancer in the world,[1] but has among the poorest survival of all cancers. The aggressive nature of the disease and the lack of early markers or effective treatment options result in the lowest 5-year survival rate (3%–7%) of all cancers in the United States.[2,3] Approximately 95% of PCs are ductal adenocarcinomas. Tobacco smoking is the main risk factor for PC and explains approximately

From the *Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain; †Department of Epidemiology, Shanghai Cancer Institute and Jiao Tong University, Shanghai, China; ‡Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY; §Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI; ||Public Health, Women's and Children's Hospital, Adelaide, SA, Australia; ¶University of California, San Francisco, San Francisco, CA; #National Institute for Public Health and the Environment (RIVM), Bilthoven; **Department of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, The Netherlands; ††Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK; ‡‡Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; §§Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Institute and MF MU, Brno, Czech Republic; ||||University Health Network, Department of Surgery, University of Toronto, Toronto, Canada; ¶¶Institute of Hygiene and Epidemiology, 1st Faculty of Medicine, Charles University in Prague, Prague; ##Department of Preventive Medicine, Faculty of Medicine, Palacky University, Olomouc, Czech Republic; ***National Cancer Institute, Bethesda, MD; †††Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; ‡‡‡Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy; §§§Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, University of Athens, Greece; ||||||Department of Epidemiology, Harvard School of Public Health, Boston, MA; ¶¶¶M.D. Anderson Cancer Center, University of Texas, Houston, TX; ###Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; ****Unit of Epidemiology and Biostatistics, CRO Aviano, National Cancer Institute, IRCCS, Aviano, Italy; ††††Cancer Center and Institute of Oncology, Warsaw, Poland; and ‡‡‡‡Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT.

Received for publication September 1, 2015; accepted February 22, 2016.

Address correspondence to: Eric J. Duell, MS, PhD, Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Avda Gran Via 199-203, 08908 L'Hospitalet de Llobregat, Barcelona, Spain (e-mail: eduell@iconcologia.net).

This work was supported by the Red Temática de Investigación Cooperativa en Cáncer (RTICC) [Rd06/0020/0091 and Rd12/0036/0018] and by the Generalitat de Catalunya. The Central Europe study was supported by the Grant Agency of Ministry of Health of the Czech Republic (IGA MZ ČR 8090-3 and 9422-3). The center of Brno, Czech Republic—within the Central Europe study—was supported by MH CZ - DRO (MMCI, 00209805) and RECAMO CZ.1.05/2.1.00/03.0101. The Italy and Milan studies were supported by the Italian Association for Cancer Research (AIRC grant 10068), and the Italian Foundation for Cancer Research (FIRC). The MD Anderson Cancer Center study was supported by the NIH Research Project Grant Program (R01 CA098380). The Pancreatic Cancer Family Registry at the Memorial Sloan Kettering Cancer Center (MSKCC) was supported by the Prevention, Control, and Population Research Goldstein Award; the Society of MSKCC; and the Geoffrey Beene Cancer Research Fund. The University of California, San Francisco (UCSF) study work was supported in part by National Cancer Institute grants CA059706, CA108370, CA109767, CA089726, CA098889, and by the Rombauer Pancreatic Cancer Research Fund. Cancer incidence data collection in the UCSF study was supported by the California Department of Public Health, the National Cancer Institute's Surveillance, Epidemiology and End Results Program contract N01-PC-35136 awarded to the Northern California Cancer Center. The Montreal investigation in the Surveillance of Environmental Aspects Related to Cancer in Humans study was supported by the Cancer Research Society, the Toronto contribution was supported by the National Cancer Institute of Canada, and the Netherlands contribution was supported by the Dutch Ministry of Public Health, Welfare and Sports (formerly Welfare, Health and Culture). The Shanghai Pancreas Cancer Study (study II) was supported by U.S. National Institute of Health grant 5R01 CA114421, by grants from the Science and Technology Commission of Shanghai Municipality, and from the Shanghai Cancer Institute (SB10-06). In all of the study support, the sponsors had no roles in the collection, analysis, or interpretation of study data.

The authors declare no conflict of interest.

Harvey A. Risch and Eric J. Duell contributed equally to this work.

DOI: 10.1097/MPA.0000000000000635

20% of the risk in a population where prevalence of smoking is 30%.[4,5] ABO non-O blood group, obesity, long-term type 2 diabetes, family history of PC, histories of pancreatitis and possibly heavy alcohol consumption, familial rare inherited mutations in *BRCA2*, *p16*, and other genes, and common variants in at least 8 genetic loci are the other known PC risk factors.[6–8]

Pancreatic cancer incidence is somewhat higher in men than in women. In the United States, between 2007 and 2011, the sex ratio ranged from 1.3 (for ages 40–44 years), to 1.1 (for ages 85 years and older).[9] In Europe, the estimated sex ratio in 2012 is highest at 1.9, for ages 40 to 44 years, and is 1.1 at ages 75 years and older.[10] Some studies, using castrated rats, showed that administration of sex steroids inhibits the development and growth of preneoplastic lesions of the pancreas.[11,12] Motivated by these observations, and under the hypothesis that greater exposure to female sex hormones (through early menarche, later menopause, high number of pregnancies, and having a history of hormone use) decreases the risk of PC, several epidemiological studies have examined possible risk associations with menstrual and reproductive factors, and hormone use, but with inconsistent results. A review paper on reproductive factors and PC,[13] 2 meta-analyses on parity,[14,15] and a recent meta-analysis[16] attempted to make clear the relations between these factors and PC risk. Comparing and summarizing previous evidence, however, is not a simple task. Inconsistencies in results may arise from different categorization and reference categories of exposure variables, different adjustment or confounding variables, and various study designs and target populations. Further, many of the previous studies were limited by small numbers of cases, and some were limited by inadequate adjustment for smoking, the primary risk factor for PC.

The aim of the present study was to assess whether menstrual or reproductive factors or hormone use are associated with risk of developing PC. Pooled individual analyses of 11 case–control studies in the Pancreatic Cancer Case–Control Consortium (PanC4) allowed us to obtain precise estimates of risks and to analyze the associations in detail.

MATERIALS AND METHODS

Studies

Eleven case–control studies with information on menstrual and reproductive factors and hormone use were available within the PanC4.[17–26] At a minimum, the studies were able to provide information on age at menarche and age at menopause. The total number of women available in the combined data set was 2838 with PC and 4748 controls. The Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH), Toronto and Shanghai-I studies included proxy responders, accounting for 14.5% of the case and 4.4% of the control women (Table 1).

Exposure Variables

Questions about menstrual and reproductive factors and exogenous hormone were generally similar across all the studies; however, full harmonization of the data was performed with the collaboration of the study investigators. Variables included in the analysis were the following: reported age at menarche, age at menopause, type of menopause (natural or surgical), history of oophorectomy, hysterectomy, age at hysterectomy, number of pregnancies, number of births (including live and stillbirths), age at first birth, number of abortions (including induced and spontaneous), history of oral contraceptive (OC) use, duration of OC use, use of menopause hormone replacement therapy (HRT), and duration of HRT. We also calculated the cumulative lifetime number of menstrual cycles, by adapting the index proposed by Chavez-MacGregor et al[27] as follows: for postmenopausal women, we calculated the difference between age at menopause and age at menarche; for each birth and stillbirth, we subtracted cycles during 36 weeks, and 12 weeks for each abortion. Menstrual cycles absent while under OC use were assumed to last 28 days duration. For premenopausal or perimenopausal women, we used age at recruitment instead of age at menopause. Missing age at menopause was imputed using the study-specific mean age at menopause or in case of both ovaries removed, the age at surgery.

Statistical Analysis

Two-stage models were used to estimate pooled odds ratios (ORs) between menstrual and reproductive factors, hormone use, and PC risk. At the first stage, for each study, the association between each factor and PC risk was assessed by estimating the OR and 95% confidence interval (CI) using study-specific logistic regression models.[28] All models in the first step were adjusted by age (<45, 45–49, 50–54, 55–59, 60–64, 65–69, 70–75, ≥75 years), education (≤8th grade, 9th–11th grade, 12th grade or high school graduates, some college or college graduate, ≥1 year of graduate school), usual body mass index (BMI, <20, 20 to <25, 25 to <30, ≥30 kg/m^2), history of nongestational diabetes mellitus, cigarette smoking (never-smokers, current smokers < 20 cigarettes/d, current smokers ≥20 cigarettes/d, ex-smokers < 10 years, ex-smokers ≥10 years), alcohol (no information available, 0 to <1 drink/d, 1 to < 4 drinks/d, ≥4 drinks/d), race (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, American Indian/Alaskan native) and center (for multicentric studies). History of pancreatitis was not included as an adjustment covariate in the present analysis because some of the studies observed no controls with this exposure. To account for possible differences in hormone levels during pregnancies, number of pregnancies was included when lifetime cumulative cycles was evaluated. At the second stage, pooled effect estimates across studies were calculated using random effects meta-analysis.[29] To evaluate study-based heterogeneity, we calculated the χ^2 statistic and the index I^2.[30] Galbraith plots were used to examine sources of heterogeneity,[31] and sensitivity analyses excluding the study/studies identified with Galbraith plots were performed to evaluate study influence on pooled ORs. Studies that contributed significantly to heterogeneity in the pooled estimates for reproductive factors were excluded from the analysis of that factor. To account for all sources of hormone exposure at the same time, mutually adjusted models were evaluated after possible collinearity between exposure variables was assessed. Effect-measure modification by tobacco use (never, current, former), BMI (under + normal weight vs obese + overweight), and histories of diabetes were evaluated using the likelihood-ratio statistic. All analyses were additionally examined restricted to postmenopausal women and to never smokers. Finally, sensitivity analyses excluding the proxy respondents and stratifying by source of control participants (population- or hospital-based) from the mutually adjusted models were also performed.

RESULTS

Table 1 gives study characteristics and distributions of the core variables according to PC status. Case subjects were older than controls, reported higher BMIs, and were more likely to be current or ex-smokers. Greater proportions of women who had

TABLE 1. Studies and Core Variables by Case and Control Status in PanC4 Women

		Cases, n (%)	Controls, n (%)	Source of Controls	Type of Interview
PC		2838 (37.4)	4748 (62.6)		
Study	Principal investigator				
North America					
MDACC (2004–2008)	Li, D	257 (9.1)	288 (6.1)	Hospital visitors	Direct
MSKCC (2003–2010)	Olson, S	424 (14.9)	242 (5.1)	Hospital visitors	Direct
Toronto (2003–2006)	Gallinger, S	217 (7.7) (6.5% proxies)	134 (2.8)	Population	Direct/proxy (4%)
UCSF (1995–1999)	Bracci, PM	237 (8.4)	818 (17.2)	Population	Direct
Europe					
Central Europe study; Poland, Czech Republic, Slovakia (2004–2009)	Scélo, G; Holcatova, I	361 (12.7)	435 (9.2)	Population	Direct
Greece (1990–1992)	Lagiou, P	66 (2.3)	132 (2.8)	Hospital, hospital visitors	Direct
Milan (1982–1999)	La Vecchia, C	133 (4.7)	409 (8.6)	Hospital	Direct
Italy (1991–2008)	Serraino, D	148 (5.2)	304 (6.4)	Hospital	Direct
China					
Shanghai-I (1990–1993)	Ji, B-T	187 (6.6) (29.4% proxies)	701 (14.8) (5.6% proxies)	Population	Direct/proxy (10.6%)
Shanghai-II (2006–2011)	Risch, HA; Gao, Y-T	445 (15.7)	464 (9.8)	Population	Direct
International					
SEARCH; Canada, The Netherlands, Poland, Australia (1983–1989)	Bueno-de-Mesquita, HB; Miller, AB; Baghurst, PA; Zatonski, W	363 (12.8) (58.4% proxies)	821 (17.3) (16.8% proxies)	Population	Direct/proxy (29.6%)
Age, y					
<45		112 (4.0)	329 (6.9)		
45–49		148 (5.2)	282 (5.9)		
50–54		228 (8.0)	488 (10.3)		
55–59		371 (13.1)	675 (14.2)		
60–64		466 (16.4)	746 (15.7)		
65–69		544 (19.2)	832 (17.5)		
70–75		504 (17.8)	778 (16.4)		
≥75		465 (16.4)	618 (13.0)		
Race					
Non-Hispanic white		1555 (67.6)	2839 (65.6)		
Non-Hispanic black		46 (2.0)	54 (1.2)		
Hispanic		18 (0.8)	53 (1.2)		
Asian/Pacific Islander		665 (28.9)	1221 (28.2)		
American Indian/Alaskan native		2 (0.1)	3 (0.1)		
Other		12 (0.5)	24 (0.6)		
Missing		1 (0.04)	133 (3.1)		
Education					
≤8th grade		833 (29.4)	1708 (36.0)		
9th–11th grade		455 (16.0)	715 (15.1)		
12th grade/high school graduate		607 (21.4)	850 (17.9)		
Some college/college graduate		581 (20.5)	990 (20.9)		
≥1 y of graduate school		341 (12.0)	454 (9.6)		
Missing		21 (0.7)	31 (0.7)		

(Continued on next page)

TABLE 1. *(Continued)*

	Cases, n (%)	Controls, n (%)	Source of Controls	Type of Interview
BMI, kg/m^2				
<20	319 (11.2)	623 (13.1)		
≥20 to <25	1298 (45.7)	2361 (49.7)		
≥25 to <30	801 (28.2)	1204 (25.4)		
≥30	391 (13.8)	511 (10.8)		
Missing	29 (1.0)	49 (1.0)		
Tobacco smoking				
Never	1730 (61.0)	3250 (68.5)		
Current smokers (cigarettes/d)				
<20	335 (11.8)	486 (10.2)		
≥20	142 (5.0)	113 (2.4)		
Ex-smokers (years since quitting)				
<10	141 (5.0)	223 (4.7)		
≥10	417 (14.7)	622 (13.1)		
Missing	73 (2.6)	54 (1.1)		
Alcohol drinking (drinks/d)*				
0 to <1	1675 (69.4)	3367 (74.7)		
1 to <4	317 (13.1)	690 (15.3)		
≥4	104 (4.3)	180 (4.0)		
Missing	318 (13.2)	269 (6.0)		
History of diabetes				
No	2269 (80.0)	4391 (92.5)		
Yes	561 (19.8)	357 (7.5)		
Missing	8 (0.3)	0 (0.0)		
History of pancreatitis†				
No	1987 (73.9)	3912 (88.0)		
Yes	123 (4.6)	49 (1.1)		
Missing	580 (21.6)	483 (10.9)		

*Information not available in the MSKCC study.

†Information not available in the Italian study.

MDACC indicates MD Anderson Cancer Center; MSKCC, Memorial Sloan Kettering Cancer Center; SEARCH, Surveillance of Environmental Aspects Related to Cancer in Humans; UCSF, University of California, San Francisco.

had diabetes or pancreatitis were observed among cases than in controls.

To account for possible differences in the exposure variables by race/ethnicity, distributions were evaluated and only minor differences in age at menarche were observed between Asian and non-Asian ethnicities; however, these differences were not statistically significant (data not shown).

All models of menstrual and reproductive factors and hormone use variables were adjusted for attained age, education, race, usual BMI, cigarette smoking, histories of diabetes and use of alcohol, and center. A statistically significant inverse association was observed in women who reported having had hysterectomy (OR, 0.78; 95% CI, 0.67–0.91), remaining consistent in postmenopausal and never-smoking women (Table 2). Earlier hysterectomy (≤41 years) showed nonsignificant inverse associations with PC risk ($OR_{\leq 37 vs > 52}$, 0.87; 0.53–1.42; $OR_{>37 \& \leq 41 vs > 52}$, 0.62; 0.37–1.05) (Table 2). The observed OR for HRT users was 0.82 (95% CI, 0.68–0.98) (Table 2).

Nonsignificant ORs below unity were observed in women who had later menarche ($OR_{>14 vs < 12}$, 0.85; 95% CI, 0.66–1.08), bilateral oophorectomy (OR, 0.83; 95% CI, 0.64–1.06), used OC (OR, 0.83; 95% CI, 0.69–1.01), reported long-term use of HRT ($OR_{\geq 36 vs \text{non-users}}$, 0.81; 95% CI, 0.66–1.00), or had high number of menstrual cycles ($OR_{>455 vs \leq 333}$, 0.74; 95% CI, 0.54–1.06) (Table 2). Although the association of high number of menstrual cycles was nonsignificant, in the subset of never-smokers, the pooled OR was 0.68 (95% CI, 0.60–0.91) (Table 2). An elevated but nonsignificant OR was observed in women with later menopause ($OR_{\geq 55 vs \leq 39}$, 1.32; 95% CI, 0.93–1.87) (P trend = 0.63). Number of pregnancies, number of births, and age at first birth showed no associations with PC risk (Table 2). We also evaluated a mutually adjusted model including all sources of hormone exposure: exogenous hormones, lifetime cumulative menstrual cycles (as a summary variable for endogenous hormone exposure); and gynecological surgery: hysterectomy and oophorectomy. Because not all the studies collected all the information mentioned previously, 2 separate models were analyzed. Studies that contributed significantly to heterogeneity in the pooled estimates in Table 2 were excluded for the mutually adjusted model. The first model included lifetime cumulative menstrual cycles, HRT, and OC use; the studies that provided information were MSKCC, Central Europe and Shanghai-II. A nonsignificant pooled OR below unity was observed in women with more than 455 cycles compared with women with at most 333 cycles ($OR_{>455 vs \leq 333}$, 0.75; 95% CI, 0.51–1.12); the pooled OR for HRT users was 0.87 (95% CI, 0.55–1.24). A null effect was observed for OC users (model 1;

TABLE 2. Menstrual, Reproductive Factors, and Hormone Use in Relation to PC Risk in PanC4 Women

	Cases, n (%)	Controls, n (%)	Pooled OR (95% CI)*	Pooled OR (95% CI)* Postmenopausal	Pooled OR (95% CI)† Never Smokers
Menstrual factors					
Age at menarche, y					
<12‡	343 (12.1)	571 (12.0)	0.98 (0.79–1.21)	0.96 (0.76–1.21)	0.91 (0.68–1.21)
12	467 (16.5)	783 (16.5)	Reference	Reference	Reference
13	634 (22.3)	949 (20.0)	1.03 (0.86–1.24)	1.05 (0.87–1.26)	1.01 (0.80–1.29)
14	475 (16.7)	871 (18.3)	0.92 (0.72–1.17)	0.92 (0.73–1.17)	0.84 (0.62–1.12)
>14	734 (25.9)	1441 (30.4)	0.85 (0.66–1.08)	0.85 (0.66–1.10)	0.85 (0.65–1.01)
Missing	185 (6.5)	133 (2.8)			
Type of menopause§‖					
Natural menopause	2053 (78.6)	3214 (78.7)		Reference	Reference
Surgical menopause	504 (19.3)	832 (20.4)		0.91 (0.77–1.08)	0.91 (0.74–1.11)
Missing	56 (2.1)	39 (0.9)			
Age at menopause, y‖					
≤39	209 (7.7)	352 (8.1)		Reference	Reference
40–44	290 (10.7)	492 (11.3)		0.99 (0.75–1.31)	1.19 (0.81–1.77)
45–49	704 (25.9)	1120 (25.8)		1.26 (0.98–1.62)	1.41 (0.92–2.15)
50–54	1013 (37.3)	1495 (34.4)		1.27 (0.97–1.66)	1.46 (0.96–2.20)
≥55	243 (8.9)	353 (8.1)		1.32 (0.93–1.87)	1.58 (0.86–2.88)
Missing	255 (9.4)	532 (12.3)			
Lifetime cumulative menstrual cycles (cycles)¶#					
≤333	239 (14.1)	474 (18.6)	Reference	Reference	Reference
>333–383	329 (19.4)	552 (21.6)	1.01 (0.74–1.38)	0.96 (0.75–1.24)	1.01 (0.62–1.63)
>383–419	378 (22.3)	536 (21.0)	1.13 (0.87–1.46)	1.08 (0.83–1.40)	1.07 (0.70–1.63)
>419–455	379 (22.3)	502 (19.7)	0.98 (0.75–1.29)	0.98 (0.74–1.28)	1.03 (0.75–1.43)
>455	336 (19.8)	468 (18.3)	0.74 (0.54–1.03)	0.75 (0.53–1.06)	0.68 (0.48–0.97)
Missing	37 (2.2)	23 (0.9)			
Oophorectomy**					
No	1512 (75.3)	2309 (75.9)	Reference	Reference	Reference
1 ovary removed	126 (6.3)	177 (5.8)	0.96 (0.72–1.27)	0.96 (0.71–1.29)	0.99 (0.67–1.45)
2 ovaries removed	279 (13.9)	442 (14.5)	0.83 (0.64–1.06)	0.84 (0.65–1.08)	0.77 (0.58–1.02)
Missing	90 (4.5)	114 (3.8)			
Hysterectomy**					
No	1361 (67.8)	1930 (63.5)	Reference	Reference	Reference
Yes	562 (28.0)	925 (30.4)	0.78 (0.67–0.91)	0.82 (0.70–0.97)	0.74 (0.60–0.91)
Missing	84 (4.2)	187 (6.2)			
Age at hysterectomy, y**††					
≤37	128 (24.3)	181 (18.7)	0.87 (0.53–1.42)	0.96 (0.57–1.62)	0.86 (0.48–1.55)
>37–41	64 (12.1)	158 (16.3)	0.62 (0.37–1.05)	0.66 (0.38–1.14)	0.51 (0.26–0.98)
>41–46	109 (20.7)	167 (17.2)	1.01 (0.53–1.91)	1.01 (0.51–2.01)	1.40 (0.79–2.47)
>46–52	90 (17.1)	163 (16.8)	0.95 (0.58–1.54)	1.01 (0.60–1.70)	0.84 (0.47–1.51)
>52	83 (15.8)	150 (15.5)	Reference	Reference	Reference
Missing	53 (10.1)	151 (15.6)			
Hysterectomy and oophorectomy**					
None of them	1265 (63.0)	1830 (60.2)	Reference	Reference	Reference
Oophorectomy	54 (2.7)	73 (2.40)	0.91 (0.60–1.39)	0.81 (0.62–1.05)	0.83 (0.63–1.08)
Hysterectomy	206 (10.3)	366 (12.0)	0.77 (0.60–1.00)	0.85 (0.64–1.14)	0.83 (0.61–1.13)
Oophorectomy and hysterectomy	348 (17.3)	540 (17.8)	0.79 (0.62–1.00)	0.85 (0.55–1.30)	0.73 (0.33–1.64)
Missing	134 (6.7)	233 (7.7)			
Reproductive factors					
No. pregnancies‡‡					
Never pregnant	233 (9.0)	461 (10.3)	Reference	Reference	Reference
1	294 (11.4)	536 (12.0)	1.05 (0.77–1.43)	1.01 (0.73–1.40)	1.06 (0.77–1.45)
2	641 (24.8)	1043 (23.4)	1.25 (0.86–1.83)	1.31 (0.89–1.95)	1.35 (0.87–1.68)
3	562 (21.8)	885 (19.8)	1.16 (0.92–1.45)	1.19 (0.93–1.52)	1.22 (0.89–1.68)
≥4	808 (31.3)	1501 (33.6)	1.10 (0.79–1.52)	1.13 (0.80–1.59)	1.23 (0.83–1.82)
Missing	43 (1.7)	36 (0.8)			
No. births (live births and stillbirths)‡‡					
Never pregnant	175 (6.8)	258 (5.8)	0.93 (0.62–1.39)	0.89 (0.58–1.37)	0.83 (0.60–1.16)

(Continued on next page)

TABLE 2. *(Continued)*

	Cases, n (%)	Controls, n (%)	Pooled OR (95% CI)*	Pooled OR (95% CI)* Postmenopausal	Pooled OR (95% CI)† Never Smokers
Nulliparous	32 (1.2)	94 (2.1)	0.49 (0.29–0.83)	0.47 (0.28–0.81)	0.50 (0.22–1.13)
1	493 (19.1)	811 (18.2)	Reference	Reference	reference
2	788 (30.5)	1347 (30.2)	0.92 (0.77–1.10)	0.94 (0.78–1.15)	0.85 (0.70–1.03)
3	518 (20.1)	799 (17.9)	1.06 (0.86–1.29)	1.05 (0.83–1.32)	1.02 (0.82–1.26)
≥4	473 (18.3)	895 (20.1)	1.05 (0.83–1.32)	1.05 (0.81–1.37)	0.94 (0.74–1.18)
Missing	102 (4.0)	256 (5.7)			
Age at first birth, y‡‡§§					
<20	214 (11.0)	370 (10.1)	Reference	Reference	Reference
20–23	656 (33.7)	1185 (32.3)	0.98 (0.78–1.24)	1.00 (0.75–1.35)	1.02 (0.75–1.39)
24–27	509 (26.2)	1034 (28.2)	0.90 (0.70–1.15)	0.97 (0.69–1.35)	0.88 (0.63–1.22)
>27	455 (23.4)	801 (21.8)	1.01 (0.65–1.55)	1.04 (0.64–1.69)	1.07 (0.76–1.52)
Missing	110 (5.7)	277 (7.6)			
No. abortions (induced and spontaneous)‡‡					
Never pregnant	175 (6.9)	258 (5.8)	Reference	Reference	Reference
0	1361 (52.7)	2204 (49.4)	1.26 (0.82–1.96)	1.40 (0.90–2.07)	1.40 (0.91–2.41)
1	562 (21.8)	1010 (22.7)	0.98 (0.75–1.27)	1.09 (0.80–1.48)	1.03 (0.67–1.60)
2	259 (10.0)	475 (10.7)	0.85 (0.55–1.33)	0.99 (0.60–1.64)	0.97 (0.53–1.80)
≥3	122 (4.7)	257 (5.8)	0.88 (0.58–1.35)	1.06 (0.68–1.65)	0.93 (0.51–1.69)
Missing	102 (3.9)	256 (5.7)			
Exogenous hormone use					
OC use‖‖					
Non-user	1863 (67.2)	3266 (70.8)	Reference	Reference	Reference
Yes	660 (23.8)	1177 (25.5)	0.83 (0.69–1.01)	0.82 (0.65–1.02)	0.86 (0.67–1.09)
Missing	249 (9.0)	173 (3.8)			
OC duration, mo‖‖¶¶##					
Non-user	1568 (73.8)	2574 (71.3)	Reference	Reference	Reference
<12	78 (3.7)	176 (4.9)	0.77 (0.49–1.21)	0.79 (0.52–1.19)	0.76 (0.41–1.40)
12–<36	131 (6.2)	225 (6.2)	0.90 (0.67–1.20)	0.88 (0.65–1.19)	1.30 (0.87–1.94)
≥36	332 (15.6)	627 (17.4)	0.84 (0.67–1.06)	0.84 (0.63–1.11)	0.85 (0.64–1.13)
Missing	15 (0.7)	9 (0.25)			
HRT use‖‖***					
No	1395 (65.2)	2251 (65.2)	Reference	Reference	Reference
Yes	632 (29.5)	1105 (32.0)	0.82 (0.68–0.98)	0.79 (0.65–0.97)	0.78 (0.58–1.05)
Missing	113 (5.3)	95 (2.8)			
HRT duration, mo‖‖†††					
Non-user	1395 (68.8)	2251 (67.1)	Reference	Reference	Reference
<12	95 (4.7)	184 (5.5)	0.86 (0.55–1.33)	0.88 (0.56–1.38)	0.92 (0.61–1.39)
12 to <36	128 (6.3)	182 (5.4)	1.04 (0.72–1.49)	1.04 (0.71–1.53)	0.94 (0.61–1.46)
≥36	393 (19.4)	703 (21.0)	0.81 (0.66–1.00)	0.80 (0.63–1.01)	0.75 (0.56–1.00)
Missing	16 (0.8)	36 (1.1)			

*Adjusted for: age in 5-year categories, education, BMI, tobacco smoking, diabetes (yes/no), alcohol, and center.

†Adjusted for: age in 5-year categories, education, BMI, diabetes (yes/no), alcohol, and center.

‡Shanghai-I was not included in this category because there were 0 cases and 2 controls.

§Information not available in Shanghai-I study.

‖In postmenopausal women.

¶Information not available in MDACC, SEARCH, Toronto and Greek studies. UCSF was excluded due to heterogeneity (I^2 = 62%) and its influence in the pooled estimates.

#Adjusted for age in 5-year categories, education, BMI, tobacco smoking, diabetes (yes/no), alcohol, center, and number of pregnancies.

**Information not available in the Milan, Greek, Shanghai-I, and Shanghai-II studies.

††Central Europe study excluded due to high heterogeneity (I^2= 53.4%) and its influence in the pooled estimations.

‡‡Information on parity not available in the MDACC study.

§§In parous women.

‖‖Information not available in the Greek study.

¶¶Shanghai I study was not included due to the proportion of non-users and short-users (<6 months) was ≥95%.

##Toronto study was not included due to the lack of controls in the highest category.

***Shanghai-I study was not included due to the proportion of non-users was ≥95%. Italy + Milan studies were excluded due to heterogeneity (I^2 = 58.2%) and its influence in the pooled estimates.

†††Milan, Italy, Shanghai-I and Shanghai-II were not included due to the proportion of non-users and short-users (<6 months) was ≥90%.

TABLE 3. Mutually Adjusted Models for Cumulative Number of Menstrual Cycles, Hormone Use, Gynecological Surgery, and PC Risk in Panc4 Women

		Cases/Controls	Pooled OR (95% CI)	Pooled OR (95% CI) Postmenopausal	Pooled OR (95% CI) Never Smokers
Model 1*†	Lifetime cumulative menstrual cycles (cycles)				
	≤333	161/135	Reference	Reference	Reference
	>333–383	208/215	0.81 (0.58–1.14)	0.81 (0.58–1.15)	0.71 (0.48–1.06)
	>383–419	273/247	0.93 (0.66–1.31)	0.94 (0.67–1.32)	0.81 (0.54–1.21)
	>419–455	288/273	0.85 (0.60–1.19)	0.85 (0.60–1.20)	0.79 (0.52–1.18)
	>455	267/252	0.75 (0.51–1.12)	0.76 (0.52–1.11)	0.65 (0.42–1.01)
	Missing	33/19			
	HRT use				
	No	995/912	Reference	Reference	Reference
	Yes	185/166	0.87 (0.55–1.39)	0.87 (0.55–1.39)	0.87 (0.53–1.42)
	Missing	60/63			
	OC use				
	Non-user	796/785	Reference	Reference	Reference
	Yes	231/223	0.95 (0.72–1.24)	0.94 (0.72–1.23)	0.95 (0.68–1.32)
	Missing	203/133			
Model 2‡§	Hysterectomy and HRT use				
	Neither	865/1114	Reference	Reference	Reference
	HRT use alone	303/492	0.84 (0.64–1.10)	0.84 (0.63–1.12)	0.73 (0.46–1.15)
	Hysterectomy alone	234/323	0.70 (0.54–0.92)	0.80 (0.60–1.07)	0.57 (0.39–0.83)
	Hysterectomy and HRT use	287/534	0.64 (0.48–0.84)	0.65 (0.48–0.88)	0.55 (0.37–0.83)
	Missing	170/275			
	Oophorectomy				
	No	1611/1144	Reference	Reference	Reference
	1 ovary removed	113/147	1.13 (0.81–1.58)	1.08 (0.77–1.53)	1.34 (0.82–2.19)
	2 ovaries removed	269/413	1.12 (0.83–1.52)	1.09 (0.80–1.48)	1.13 (0.77–1.66)
	Missing	88/101			
	OC use				
	Non-user	1046/1576	Reference	Reference	Reference
	Yes	565/990	0.78 (0.61–0.99)	0.76 (0.57–1.01)	0.83 (0.59–1.15)
	Missing	248/172			

*Adjusted for: age in 5-year categories, education, BMI, tobacco smoking, diabetes (yes/no), alcohol, center, and number of pregnancies. Never smokers model did not include tobacco smoking variable.

†Studies included MSKCC, Central Europe, and Shanghai-II.

‡Adjusted for: age in 5-year categories, education, BMI, tobacco smoking, diabetes (yes/no), alcohol and center. Never smokers model did not include tobacco smoking variable.

§Studies included MDACC, MSKCC, Toronto, UCSF, Central Europe, and SEARCH.

Table 3). The second mutually adjusted model included MDACC, MSKCC, Toronto, UCSF, Central Europe, and SEARCH studies. For this model, the joint effect of hysterectomy and HRT use was evaluated according to the following categories: use of neither, HRT use alone, hysterectomy alone, and both hysterectomy plus HRT use (model 2; Table 3). This joint effect variable was evaluated because the frequency of HRT use was 3 times as high in women who had had hysterectomies than not (data not shown). History of oophorectomy and OC use were also included in the model (model 2; Table 3). The joint effect analysis of hysterectomy and HRT use showed that HRT use without hysterectomy conveyed a nonsignificant inverse association with PC ($OR_{HRT\,vs\,none}$, 0.84; 95% CI, 0.64–1.10), whereas a significant inverse association with PC risk was observed in women with hysterectomy without HRT use ($OR_{hysterectomy\,vs\,none}$, 0.70; 95% CI, 0.54–0.92) which was somewhat lower ($OR_{hysterectomy+HRT\,vs\,neither}$, 0.64; 95% CI, 0.48–0.84) for women also taking HRT. No statistically significant association was observed in relation to oophorectomy. A sensitivity analysis was performed, where the joint effect was considered only when HRT use started at the same time or after the hysterectomy; however, the results did not change (data not shown). A forest plot of the study-specific and the pooled ORs for the joint effect of hysterectomy and use of HRT and PC risk is presented in Figure 1.

We found no evidence for effect-measure modification of the relation between each factor and PC risk by cigarette smoking, BMI, and history of diabetes (all likelihood ratio statistic $P > 0.05$) (data not shown). Sensitivity analyses in never-smoking women and in postmenopausal women were performed, but in general, except as noted previously, results did not change (Tables 2 and 3). No major differences in pooled OR estimates were observed when

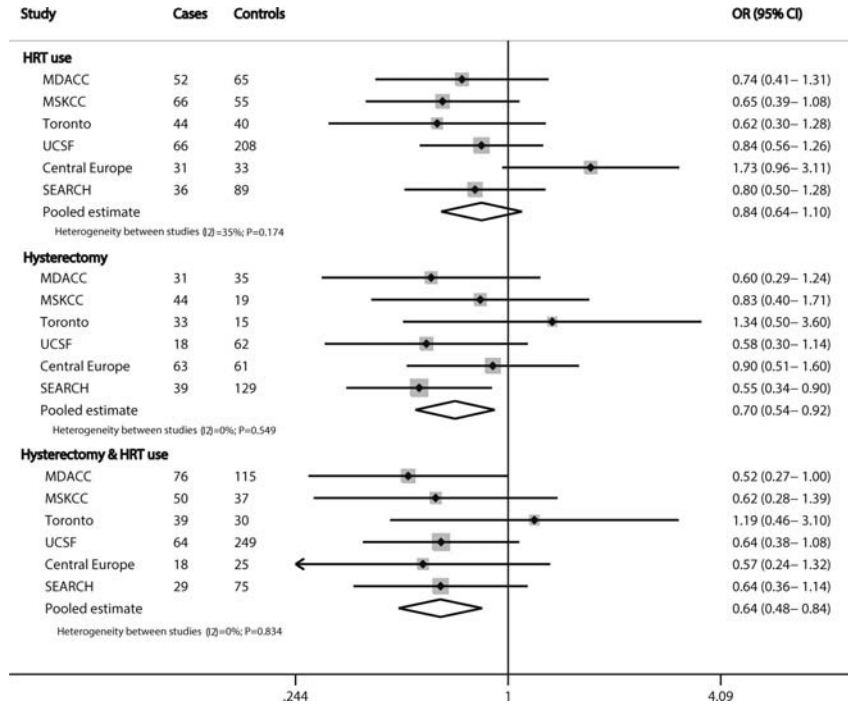


FIGURE 1. Study-specific and pooled OR estimates for the associations of hysterectomy and HRT use with risk of PC in PanC4 women (model 2; Table 3). Adjusted for: age in 5-year categories, education, BMI, cigarette smoking, diabetes, alcohol, center, oophorectomy, and OC use. Box sizes are weighted by the inverse of the variance.

proxy-respondents were excluded from the mutually adjusted models or when models were stratified by the source of control participants (data not shown).

DISCUSSION

The present pooled data analysis of 11 case–control studies, including 2838 PC case women and 4748 controls, allowed us to estimate more precisely possible relations between menstrual and reproductive factors, hormone use, and PC risk. Our results suggest that undergoing hysterectomy may significantly reduce the risk of developing PC by 22%.

A nonstatistically significant OR below unity was observed with high lifetime cumulative number of menstrual cycles (>455 cycles). When the analysis was restricted to never-smokers, this association became statistically significant. Lifetime cumulative menstrual cycles are an index for total exposure to endogenous hormones. Because hormonal levels differ during pregnancy, we subtracted from the calculation 36 and 12 weeks for each birth and abortion, respectively, and included the number of pregnancies as an adjustment variable; and hormone use (HRT and OC) was also included in the model. Chavez-MacGregor et al[27] also excluded from the calculation a 6-week absence of cycles for women who reported lactation, and they accounted for menstrual cycle irregularity. Unfortunately, this information was not available in the included PanC4 studies. Because smoking is an important risk factor for PC and may also affect sex hormone levels,[32] we carried out additional analyses limited to never-smokers, although similar patterns of relative risk were observed. Obese and overweight persons have an increased risk of PC,[33] and high BMI is positively associated with high estrogens levels[34]; but in our analysis, different levels of BMI did not alter our OR estimations. Further analyses were also performed in postmenopausal women, and similar estimates of risk and 95% CIs were observed.

In the published literature, 7 previous studies of PC collected information on oophorectomy and hysterectomy; and all case–control studies that collected this information[18–20,35] were included in the present pooled analysis. Hysterectomy prevalence in PanC4 cases is almost 28% (American studies: 33%, European studies: 21%). In the American cohort studies, hysterectomy prevalence in PC cases is approximately 40%,[36,37] whereas in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort the prevalence was almost 15%.[38] The Iowa Women's health study (IWHS) cohort showed a statistically significant increase in risk for both hysterectomy (hazard ratio [HR], 1.37; 95% CI, 1.02–1.82) and bilateral oophorectomy (HR, 1.43; 95% CI, 1.01–2.00).[39] The EPIC cohort observed a no effect of bilateral oophorectomy and hysterectomy with ovarian conservation on PC risk.[38] The Cancer Prevention Study-II Nutrition Cohort found that hysterectomy with ovarian conservation was associated with an increased risk of PC (OR, 1.48; 95% CI, 1.03–2.14), although no effect of hysterectomy with bilateral oophorectomy on PC was observed (OR, 0.97; 95% CI, 0.69–1.37).[36] A recent meta-analysis observed[16] an inverse association in relation to hysterectomy and PC risk in case–control studies (OR, 0.77; 95% CI, 0.64–0.94) and no association in cohort studies. This result in case–control studies agreed with our general findings on hysterectomy (OR, 0.78; 95% CI, 0.67–0.91). Discrepancies between cohort studies and case–controls studies might be explained by selection bias in hospital-based (HB) case–control studies; however, we observed that the association between hysterectomy and PC was borderline in the population-based (PB) case–control studies (PB: OR, 0.84; 95% CI, 0.69–1.01; HB: OR, 0.70; 95% CI, 0.54–0.90) and results by source of controls were not considered heterogeneous (Wald statistic for heterogeneity: 1.21, $P = 0.271$).

The role of HRT in relation to PC risk is not clear. Although 2 cohort[37,40] showed nonsignificant inverse associations, one cohort

study[41] and a case–control studies[42] showed nonsignificant increases in risk. Finally, 2 studies showed relative risk estimates for HRT close to unity.[38,39] All remaining case–control studies were included in our pooled analysis.[18–20,35] None of these studies, or our pooled analyses, was able to distinguish the type of hormone therapy used, for example, whether combinations of estrogen and progestin were used, or estrogen alone. Use patterns for type of hormone therapy have changed during the past decades, with eras of estrogen alone and estrogen plus progestin.[43] The Women's Health Initiative (WHI) randomized controlled trial findings support the hypothesis that estrogen alone or estrogen plus progestin may not have the same biological effects. The WHI observed that women who reported the combination of estrogen and progestin had an increased risk of breast cancer and a nonsignificant decrease in the risk of endometrial cancer,[44] whereas taking estrogen alone did not show an increase of breast cancer in women with hysterectomy.[45] Thus, estrogen-only HRT is usually recommended for women who have had hysterectomy.[43] To evaluate the influence of changes in the consumption patterns of HRT, we analyzed the use of HRT by study year (before 2002 vs after 2002); and we observed no differences in the pooled risk estimate.

In PanC4, information on HRT type was not available; however, we did observe that the frequency of HRT use was 3 times as high among women who reported hysterectomy than not. Further, almost 57% of women started the HRT treatment at the age of the hysterectomy, and 30% after hysterectomy. We observed a 36% lower risk in comparison to women with intact uteri and who had not used HRT. It is possible that women who have had hysterectomy and used HRT may have lower PC risk because of other factors besides hysterectomy and HRT.

Some diseases, for which the treatment is hysterectomy, are directly related with increased female hormone levels. Almost 70% of American women[46] during their fertile life will have fibroids. In many cases, fibroids do not cause symptoms, however, they can cause abnormal bleeding, and pelvic pressure, for which hysterectomy is the recommended treatment.[46,47] Among the factors that can contribute to fibroid growth are elevated levels of estrogens and progesterone.[47] Further, women diagnosed with hyperplasia tend to undergo hysterectomy surgery.[48] Hyperplasia is associated with higher levels of estrogens and insufficient levels of progesterone, and may evolve to endometrial cancer.[48] Unfortunately, the specific reason for having a hysterectomy was not available in PanC4 data, so we could not verify if the observed protective effect that was shown with hysterectomy was due to underlying elevated estrogen levels in the mentioned diseases or to other factors related to having a hysterectomy.

Lifetime cumulative menstrual cycle is an index that attempts to summarize reproductive information, including information on menarche, menopause, pregnancies, OC, and lactation. Our analytical approach allowed us to evaluate the index including these factors, with the exception of lactation. Three previous studies of PC have evaluated the index, but each study used a different calculation and different adjustment variables.[38,39,49] All the studies, including our analysis, found no association between lifetime cumulative menstrual cycles and PC risk, suggesting little or no effect of female hormones on PC risk.

In light of the inconsistent results between the studies on menstrual and reproductive factors, hormone use and PC risk, it is worth noting that previous studies had diverse study designs, target populations, and confounder and adjustment variables; specifically, adjustments for smoking and alcohol. Furthermore, risk estimates for hysterectomy and for oophorectomy were inconsistent in our analysis with those provided by cohort studies,[36,38,39] possibly caused by selection bias in case–control studies.

The main weakness of our analysis is that not all of the studies collected all of the information on menstrual and reproductive factors and hormone use. Thus, mutually adjusted models could be obtained in only 4 or 6 studies, depending on the included variables, and a model that contained all collected factors in all studies was not possible. Another weakness of our analyses is that for some variables such as age at menopause, and for OC and HRT durations, we received categorical variables from some of the studies, so cut points for these variables had to be based on the received information, or the information could not be included in the calculation of lifetime cumulative menstrual cycles. Also, time periods of studies vary from the 1980s to 2011, however, low heterogeneity between studies in each pooled OR estimation was observed. Even so, the PanC4 consortium includes a large data set which allowed us to adjust for cigarette smoking, alcohol intake, and other potential confounding variables, and with sufficient power to estimate ORs across strata of major PC risk factors, for example, smoking, BMI, and diabetes.

In conclusion, our pooled analysis found no associations between age at menarche, menopause, lifetime cumulative menstrual cycles, oophorectomy, parity, history of OC use, and PC risk, but suggests that women who have had hysterectomy may be at lower risk of PC. Further investigations by type and formulation of HRT and reason for hysterectomy could clarify the role, if any, of hysterectomy in relation to PC risk.

REFERENCES

1. Worldwide Data—Cancer Facts & Figures. [World Cancer Research Fund International]. October 1, 2014. Available at: http://www.wcrf.org/int/cancer-facts-figures/worldwide-data. Accessed October 1, 2014.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65:5–29.
3. Gong Z, Holly EA, Bracci PM. Survival in population-based pancreatic cancer patients: san francisco bay area, 1995–1999. *Am J Epidemiol*. 2011; 174:1373–1381.
4. Rosato V, Polesel J, Bosetti C, et al. Population attributable risk for pancreatic cancer in northern Italy. *Pancreas*. 2015;44:216–220.
5. Iodice S, Gandini S, Maisonneuve P, et al. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. *Langenbecks Arch Surg*. 2008;393:535–545.
6. Yeo TP, Lowenfels AB. Demographics and epidemiology of pancreatic cancer. *Cancer J*. 2012;18:477–484.
7. Anderson KE, Mack TM. Silverman DT. In: Schottenfeld D, Fraumeni JF, eds. *Cancer Epidemiology and Prevention, Vol 3*. New York, NY: Oxford University Press; 2006:721–762.
8. Wolpin BM, Rizzato C, Kraft P, et al. Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. *Nat Genet*. 2014;46:994–1000.
9. Surveilleance, Epidemiology, and End Results Program. [Fast stats]. February 19, 2015. Available at: http://seer.cancer.gov/faststats/. Accessed February 19, 2015.
10. GLOBACAN 2012: Estimated Cancer Incidence, Moratality and Prevalence Worldwide in 2012. [International Agency for Research on Cancer]. February 19, 2015. Available at: http://globocan.iarc.fr/Pages/age-specific_table_sel.aspx. Accessed February 19, 2015.
11. Sumi C, Longnecker DS, Roebuck BD, et al. Inhibitory effects of estrogen and castration on the early stage of pancreatic carcinogenesis in fischer rats treated with azaserine. *Cancer Res*. 1989;49:2332–2336.
12. Sumi C, Brinck-Johnsen T, Longnecker DS. Inhibition of a transplantable pancreatic carcinoma by castration and estradiol administration in rats. *Cancer Res*. 1989;49:6687–6692.

13. Wahi MM, Shah N, Schrock CE, et al. Reproductive factors and risk of pancreatic cancer in women: a review of the literature. *Ann Epidemiol*. 2009;19:103–111.

14. Guan HB, Wu L, Wu QJ, et al. Parity and pancreatic cancer risk: a dose–response meta-analysis of epidemiologic studies. *PLoS One*. 2014; 9:e92738.

15. Zhu B, Zou L, Han J, et al. Parity and pancreatic cancer risk: evidence from a meta-analysis of twenty epidemiologic studies. *Sci Rep*. 2014;4:5313.

16. Tang B, Lv J, Li Y, et al. Relationship between female hormonal and menstrual factors and pancreatic cancer: a meta-analysis of observational studies. *Medicine (Baltimore)*. 2015;94:e177.

17. Hassan MM, Bondy ML, Wolff RA, et al. Risk factors for pancreatic cancer: case–control study. *Am J Gastroenterol*. 2007;102:2696–2707.

18. Lucenteforte E, Zucchetto A, Bosetti C, et al. Reproductive and hormonal factors and pancreatic cancer risk in women. *Pancreas*. 2011;40:460–463.

19. Duell EJ, Holly EA. Reproductive and menstrual risk factors for pancreatic cancer: a population-based study of San Francisco Bay Area women. *Am J Epidemiol*. 2005;161:741–747.

20. Duell EJ, Maisonneuve P, Baghurst PA, et al. Menstrual and reproductive factors and pancreatic cancer in the SEARCH program of the IARC. *Cancer Causes Control*. 2009;20:1757–1762.

21. Anderson LN, Cotterchio M, Gallinger S. Lifestyle, dietary, and medical history factors associated with pancreatic cancer risk in Ontario, Canada. *Cancer Causes Control*. 2009;20:825–834.

22. Olson SH, Orlow I, Simon J, et al. Allergies, variants in IL-4 and IL-4R alpha genes, and risk of pancreatic cancer. *Cancer Detect Prev*. 2007;31: 345–351.

23. Ji BT, Hatch MC, Chow WH, et al. Anthropometric and reproductive factors and the risk of pancreatic cancer: a case–control study in Shanghai, China. *Int J Cancer*. 1996;66:432–437.

24. Risch HA, Lu L, Kidd MS, et al. Helicobacter pylori seropositivities and risk of pancreatic carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2014;23:172–178.

25. Urayama KY, Holcatova I, Janout V, et al. Body mass index and body size in early adulthood and risk of pancreatic cancer in a central European multicenter case–control study. *Int J Cancer*. 2011;129:2875–2884.

26. Kalapothaki V, Tzonou A, Hsieh CC, et al. Tobacco, ethanol, coffee, pancreatitis, diabetes mellitus, and cholelithiasis as risk factors for pancreatic carcinoma. *Cancer Causes Control*. 1993;4:375–382.

27. Chavez-MacGregor M, Elias SG, Onland-Moret NC, et al. Postmenopausal breast cancer risk and cumulative number of menstrual cycles. *Cancer Epidemiol Biomarkers Prev*. 2005;14:799–804.

28. Breslow NE, Day NE. *Statistical Methods in Cancer Research. The Analysis of Case-Controls Studies, Vol 1*. Lyon, France: IARC; 1980.

29. Smith-Warner SA, Spiegelman D, Ritz J, et al. Methods for pooling results of epidemiologic studies: the pooling project of prospective studies of diet and cancer. *Am J Epidemiol*. 2006;163:1053–1064.

30. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539–1558.

31. Galbraith RF. Graphical display of estimates having differing standard errors. *Technometrics*. 1988;30:271–281.

32. Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. *Eur J Endocrinol*. 2005;152:491–499.

33. Preziosi G, Oben JA, Fusai G. Obesity and pancreatic cancer. *Surg Oncol*. 2014;23:61–71.

34. Olson MB, Shaw LJ, Kaizar EE, et al. Obesity distribution and reproductive hormone levels in women: a report from the NHLBI-sponsored WISE study. *J Womens Health (Larchmt)*. 2006;15:836–842.

35. Bueno de Mesquita HB, Maisonneuve P, Moerman CJ, et al. Anthropometric and reproductive variables and exocrine carcinoma of the pancreas: a population-based case–control study in the Netherlands. *Int J Cancer*. 1992;52:24–29.

36. Gaudet MM, Gapstur SM, Sun J, et al. Oophorectomy and hysterectomy and cancer incidence in the cancer prevention study-II nutrition cohort. *Obstet Gynecol*. 2014;123:1247–1255.

37. Lee E, Horn-Ross PL, Rull RP, et al. Reproductive factors, exogenous hormones, and pancreatic cancer risk in the CTS. *Am J Epidemiol*. 2013; 178:1403–1413.

38. Duell EJ, Travier N, Lujan-Barroso L, et al. Menstrual and reproductive factors in women, genetic variation in CYP17A1, and pancreatic cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. *Int J Cancer*. 2013;132:2164–2175.

39. Prizment AE, Anderson KE, Hong CP, et al. Pancreatic cancer incidence in relation to female reproductive factors: Iowa women's health study. *JOP*. 2007;8:16–27.

40. Navarro Silvera SA, Miller AB, Rohan TE. Hormonal and reproductive factors and pancreatic cancer risk: a prospective cohort study. *Pancreas*. 2005;30:369–374.

41. Skinner HG, Michaud DS, Colditz GA, et al. Parity, reproductive factors, and the risk of pancreatic cancer in women. *Cancer Epidemiol Biomarkers Prev*. 2003;12:433–438.

42. Lo AC, Soliman AS, El-Ghawalby N, et al. Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. *Pancreas*. 2007;35:120–129.

43. Lacey JV, Colditz GA, Schottenfeld D. In: Schottenfeld D, Fraumeni JF, eds. *Cancer Epidemiology and Prevention, Vol 3*. New York, NY: Oxford University Press; 2006:468–488.

44. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA*. 2002;288:321–333.

45. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the women's health initiative randomized controlled trial. *JAMA*. 2004;291:1701–1712.

46. NIH Fact Sheets—Uterine Fibroids. [National Institutes of Health]. 2013. Available at: http://report.nih.gov/nihfactsheets/viewfactsheet.aspx?csid=50. Accessed May 18, 2015.

47. Hysterectomy. [Medscape]. 2015. Available at: http://emedicine.medscape.com/article/267273-overview#a0103. Accessed May 18, 2015.

48. Lacey JV, Chia VM, Rush BB, et al. Incidence rates of endometrial hyperplasia, endometrial cancer and hysterectomy from 1980 to 2003 within a large prepaid health plan. *Int J Cancer*. 2012;131:1921–1929.

49. Fernandez E, La Vecchia C, D'Avanzo B, et al. Menstrual and reproductive factors and pancreatic cancer risk in women. *Int J Cancer*. 1995;62:11–14.

PAPER – III

Reproductive Factors, Exogenous Hormone Use, and Risk of B-Cell Non-Hodgkin Lymphoma in a Cohort of Women from the European Prospective Investigation Into Cancer and Nutrition

Costas L, **Lujan-Barroso L**, Benavente Y, Allen NE, Amiano P, Ardanaz E, Besson C, Boeing H, Bueno-de-Mesquita B, Cervenka I, Fortner RT, Fournier A, Gunter M, Harlid S, Huerta JM, Jerkeman M, Jirström K, Kaaks R, Karakatsani A, Khaw KT, Kotanidou A, Lund E, Masala G, Mattiello A, Melin B, Menéndez , Murphy N, Nieters A, Overvad K, Riboli E, Sacerdote C, Sánchez MJ, Schmidt JA, Sieri S, Tjønneland A, Trichipoulou A, Tumino R, Vermeulen R, Weiderpass E, de Sanjosé S, Agudo A, Casabonne D.

Am J Epidemiol (2018); 188 (2): 274-81

American Journal of Epidemiology

Vol. 188, No. 2
DOI: 10.1093/aje/kwy259
Advance Access publication:
November 27, 2018

Original Contribution

Reproductive Factors, Exogenous Hormone Use, and Risk of B-Cell Non-Hodgkin Lymphoma in a Cohort of Women From the European Prospective Investigation Into Cancer and Nutrition

Laura Costas*, Leila Lujan-Barroso, Yolanda Benavente, Naomi E. Allen, Pilar Amiano, Eva Ardanaz, Caroline Besson, Heiner Boeing, Bas Bueno-de-Mesquita, Iris Cervenka, Renée T. Fortner, Agnès Fournier, Marc Gunter, Sophia Harlid, José María Huerta, Mats Jerkeman, Karin Jirström, Rudolf Kaaks, Anna Karakatsani, Kay-Tee Khaw, Anastasia Kotanidou, Eiliv Lund, Giovanna Masala, Amalia Mattiello, Beatrice Melin, Virginia Menéndez, Neil Murphy, Alexandra Nieters, Kim Overvad, Elio Riboli, Carlotta Sacerdote, Maria-Jose Sánchez, Julie A. Schmidt, Sabina Sieri, Anne Tjønneland, Antonia Trichopoulou, Rosario Tumino, Roel Vermeulen, Elisabete Weiderpass, Silvia de Sanjosé, Antonio Agudo, and Delphine Casabonne

* Correspondence to Dr. Laura Costas, Unit of Molecular Epidemiology and Genetics in Infections and Cancer, Cancer Epidemiology Research Programme, Institut Català d'Oncologia, Av. Gran Via de l'Hospitalet 199-203, 08908 L'Hospitalet de Llobregat, Spain (e-mail: lcostas@iconcologia.net).

Initially submitted September 5, 2018; accepted for publication November 16, 2018.

The role of hormonal factors in the etiology of lymphoid neoplasms remains unclear. Previous studies have yielded conflicting results, have lacked sufficient statistical power to assess many lymphoma subtypes, or have lacked detailed information on relevant exposures. Within the European Prospective Investigation Into Cancer and Nutrition cohort, we analyzed comprehensive data on reproductive factors and exogenous hormone use collected at baseline (1992–2000) among 343,458 women, including data on 1,427 incident cases of B-cell non-Hodgkin lymphoma (NHL) and its major subtypes identified after a mean follow-up period of 14 years (through 2015). We estimated hazard ratios and 95% confidence intervals using multivariable proportional hazards modeling. Overall, we observed no statistically significant associations between parity, age at first birth, breastfeeding, oral contraceptive use, or ever use of postmenopausal hormone therapy and risk of B-cell NHL or its subtypes. Women who had undergone surgical menopause had a 51% higher risk of B-cell NHL (based on 67 cases) than women with natural menopause (hazard ratio = 1.51, 95% confidence interval: 1.17, 1.94). Given that this result may have been due to chance, our results provide little support for the hypothesis that sex hormones play a role in lymphomagenesis.

cohort studies; hormone therapy; hysterectomy; lymphoma; menopause; menstrual factors; oophorectomy; parity

Abbreviations: CI, confidence interval; EPIC, European Prospective Investigation Into Cancer and Nutrition; HR, hazard ratio; ICD-O-2, *International Classification of Diseases for Oncology, Second Edition*; NHL, non-Hodgkin lymphoma.

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of hematological malignancies, the incidence of which has risen in some Western countries since the 1970s, although it seems to have reached a plateau during the last few decades. Incidence rates of NHL are higher in men than in women for most NHL subtypes (1). Reproductive hormones interact with the immune system in numerous ways (2, 3), and women produce a more vigorous cellular and humoral response than men (4). Increasing evidence suggests a role of estrogens in hematological malignancies (5). While normal human peripheral blood cells express both estrogen receptor α and estrogen receptor β, lymphoid neoplasms express and up-regulate estrogen receptor β (6, 7). Furthermore, estrogen receptor β agonists have been shown to strongly inhibit the growth of lymphoma and leukemia cells in mice (8, 9).

Although the interaction between the endocrine and immune systems is complex, hormonal influences in NHL etiology seem biologically plausible. However, analyses that have examined the association of reproductive factors with NHL risk have been inconsistent, probably because of study limitations (10), including lack of detailed data on hormonal factors and limited statistical power to examine these associations for different NHL subtypes, which may have different etiologies (11). We therefore investigated the roles of reproductive factors and exogenous hormone use in risk of B-cell NHL using detailed data from a large cohort study, the European Prospective Investigation Into Cancer and Nutrition (EPIC).

METHODS

EPIC is an ongoing multicenter cohort study that recruited 521,324 participants between 1992 and 2000 from 23 research centers in 10 European countries. Participants were generally recruited from the general population residing in a geographic area. Exceptions were the cities of Utrecht, the Netherlands, and Florence, Italy (women participating in breast cancer screening programs); parts of the Italian and Spanish cohorts (blood donors); most of the Oxford, United Kingdom, cohort (vegetarian volunteers); and participants from France and Germany (health care insurance organizations). In France, Norway, Utrecht, and Naples, Italy, only women were enrolled (12). At recruitment, participants signed a consent form and provided information on diet and lifestyle, and anthropometric measurements were taken. Data collection procedures were centralized as those of a single study with multiple centers. Specific questionnaires for women were used to collect information on menstrual factors, reproductive history, and use of exogenous hormones (12). Participants with prevalent cancer (except nonmelanoma skin cancer) and those with missing follow-up information were excluded ($n = 29{,}332$). Men ($n = 148{,}007$) and persons with incomplete information on lifestyle factors ($n = 527$) were excluded from the present analysis.

Incident lymphoma cases were identified through population cancer registries and active follow-up (through 2015), including use of health insurance records, hospital registries, and direct contacts with participants or next of kin. Lymphoid neoplasms were initially classified according to the *International Classification of Diseases for Oncology, Second Edition* (ICD-O-2), and were then later recoded to the *International Classification of Diseases for Oncology, Third Edition*, from the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues (13). The conversion was made using an algorithm available on the Surveillance, Epidemiology, and End Results web page (http://www.seer.cancer.gov/) and involved a pathology expert and local expertise from participating EPIC centers. Cases with ICD-O-2 codes that could not be translated unequivocally into a lymphoid neoplasm diagnosis according to the World Health Organization classification system were categorized as "lymphoid neoplasm, unclassified" ("NOS"). The classification was further revised by participating centers using the InterLymph Pathology Working Group classification, which is based in the 2008 World Health Organization classification (13). We refer here to "B-cell NHL," which is equivalent to "mature B-cell neoplasms" as defined by the World Health Organization and which includes multiple myeloma and chronic lymphocytic leukemia/small lymphocytic leukemia in its definition. The total number of cases included 1,849 lymphomas, of which 1,427 were B-cell NHL, 73 were T-cell NHL, 80 were Hodgkin lymphoma, and 269 were other unclassified subtypes of lymphoma. The 1,427 B-cell NHL cases were further categorized into 302 cases of diffuse large-cell lymphoma, 264 cases of follicular lymphoma, 289 cases of chronic lymphocytic leukemia/small lymphocytic lymphoma, 387 cases of multiple myeloma, and 185 cases of other subtypes of B-cell NHL. Analyses of Hodgkin lymphoma, T-cell NHL, and other unclassified subtypes of lymphoma were not performed owing to small numbers. Thus, the present analyses were based on 343,458 women and 1,427 cases of B-cell NHL.

Information on variables included in the analysis was collected at baseline using standardized questionnaires. These variables included reported age at menarche, number of full-term pregnancies, age at first birth, breastfeeding, duration of breastfeeding, history and duration of oral contraceptive use and postmenopausal hormone therapy, menopausal status, reported age at natural menopause, oophorectomy, and hysterectomy. For the hormone therapy variables, participants were asked whether they had ever used these drugs and about the timing of use, age at starting use, total duration of use, and type of formulation (estrogen alone, progestin alone, or estrogen + progestin). Self-reported baseline menopausal status was defined as menopausal (natural cessation of menses in the last 12 months or surgical menopause due to bilateral ovariectomy), perimenopausal (no longer naturally menstruating at the time of recruitment or fewer than 9 menstrual cycles in the past 12 months), and premenopausal (regular menses or at least 9 menstrual cycles in the past 12 months).

Proportional hazards modeling was used to estimate hazard ratios and 95% confidence intervals for reproductive factors and risk of B-cell NHL and its major subtypes. Age was used as the underlying time scale, and all models stratified by age at recruitment (1 year-categories) and study center and adjusted for educational level. Body mass index, physical activity, and smoking status were not included as adjustment covariates because they did not change the risk estimates by more than 10%. The proportional hazards assumption was checked using graphical methods and a goodness-of-fit test. Additional analyses were performed by means of the Wald test statistic to assess the homogeneity of the risk between lymphoma subtypes, using the SAS macro %SUBTYPE (SAS Institute, Inc., Cary, North Carolina) (14). All analyses were performed with SAS, version 9.4.

RESULTS

The analytical cohort was followed for an average of 14 years, for a total of 4,792,436 person-years. Baseline characteristics of participants are presented in Web Table 1 (available at https://academic.oup.com/aje). Overall, age at menarche, parity, and breastfeeding (Table 1), as well as oral contraceptive use and age at natural menopause (Table 2), were not statistically significantly associated with B-cell NHL risk.

Surgical menopause was significantly associated with B-cell NHL risk as compared with natural menopause (hazard ratio (HR) = 1.51, 95% confidence interval (CI): 1.17, 1.94; Table 2).

Table 1. Risk of B-Cell Non-Hodgkin Lymphoma According to Baseline Menstrual and Reproductive Characteristics, European Prospective Investigation Into Cancer and Nutrition, 1992–2000

Characteristic	No. of Person-Years	No. of Cases[a]	HR[b]	95% CI	*P* for Trend
Age at menarche, years					0.65
<12	699,808	191	1.00	Referent	
12	978,186	251	0.92	0.76, 1.11	
13	1,194,471	372	1.06	0.89, 1.27	
14	1,002,365	326	1.02	0.85, 1.23	
>14	741,051	241	0.90	0.74, 1.10	
Missing data		46			
Parity (no. of full-term pregnancies)					0.82
0 (nulliparous)	706,477	176	1.00	Referent	
1	683,853	207	0.97	0.79, 1.19	
2	1,832,221	516	0.87	0.73, 1.04	
≥3	1,244,184	458	1.02	0.85, 1.22	
Missing data		70			
Age at first full-term pregnancy[c], years					0.91
≤20	563,887	172	1.00	Referent	
21–23	1,029,538	319	1.01	0.84, 1.22	
24–25	760,262	248	1.05	0.86, 1.28	
26–30	1,112,936	335	0.97	0.80, 1.17	
>30	392,245	125	1.08	0.85, 1.38	
Missing data		4			
Breastfeeding[c]					
Never breast-fed	541,260	138	1.00	Referent	
Ever breast-fed	3,058,525	984	1.16	0.97, 1.39	
Missing data		81			
Duration of breastfeeding[d], months					0.41
≤2 (T1)	976,473	304	1.00	Referent	
3–8 (T2)	1,005,212	304	0.91	0.77, 1.07	
≥9 (T3)	1,044,614	370	1.06	0.90, 1.26	
Missing data		6			

Abbreviations: CI, confidence interval; HR, hazard ratio; T, tertile.
[a] Numbers may not sum to totals because of missing values.
[b] All models stratified the data by center and age and adjusted the results for educational level.
[c] Among parous women (n = 1,203 cases).
[d] Among parous women who had ever breast-fed (n = 984 cases).

Accordingly, women who had undergone both hysterectomy and oophorectomy had a 26% higher risk of B-cell NHL compared with women who had not (HR = 1.26, 95% CI: 1.01, 1.56). Associations were more pronounced among women who had undergone bilateral oophorectomy (HR = 1.51, 95% CI: 1.19, 1.91) than among women who had undergone unilateral oophorectomy (HR = 0.81, 95% CI: 0.59, 1.10), as compared with women with intact ovaries (data not shown). Postmenopausal hormone therapy was not associated with B-cell NHL (HR = 1.03, 95% CI: 0.91, 1.18) overall or by formulation (estrogen alone, progestin alone, or estrogen plus progestin). Among women with no postmenopausal hormone therapy use, having a surgical menopause was still associated with greater B-cell NHL risk than having a natural menopause (HR = 1.74, 95% CI: 1.19, 2.49; data not shown).

No consistent associations were found in the analyses by lymphoma subtype (Web Tables 2 and 3). No significant heterogeneity was observed by subtype for any of the potential risk factors evaluated (data not shown). Results of analyses combining B-cell and T-cell subtypes and censoring multiple myeloma from the definition of lymphoma are provided in Web Tables 4 and 5 for comparability with previous studies; results were similar.

DISCUSSION

In this analysis of women from a large prospective cohort study, we generally observed null associations with reproductive

Table 2. Risk of B-Cell Non-Hodgkin Lymphoma According to Baseline Exogenous Hormone Use and Menopausal Factors, European Prospective Investigation Into Cancer and Nutrition, 1992–2000

Characteristic	No. of Person-Years	No. of Cases[a]	HR[b]	95% CI	*P* for Trend
Oral contraceptive use					
Never use	1,919,675	687	1.00	Referent	
Ever use	2,731,051	706	0.93	0.83, 1.05	
Missing data		34			
Duration of oral contraceptive use, years					0.16
0 (never use)	1,919,675	687	1.00	Referent	
0.2–3.9 (T1)	759,811	222	1.02	0.87, 1.20	
4.0–9.9 (T2)	879,090	206	0.90	0.76, 1.07	
≥10.0 (T3)	833,513	220	0.91	0.77, 1.07	
Missing data		92			
Menopausal status					
Premenopausal	1,693,207	256	1.19	0.92, 1.54	
Perimenopausal	918,876	249	0.93	0.77, 1.13	
Postmenopausal					
Natural	2,058,663	855	1.00	Referent	
Surgical	121,690	67	1.51	1.17, 1.94	
Missing data		0			
Age at natural menopause (n = 855), years					0.83
≤45	331,008	132	1.00	Referent	
46–50	661,418	274	1.03	0.83, 1.27	
>50	595,101	256	1.00	0.80, 1.23	
Missing data		193			
Oophorectomy and hysterectomy status					
Neither	3,253,667	885	1.00	Referent	
Oophorectomy	87,407	28	0.97	0.64, 1.42	
Hysterectomy	244,685	105	1.17	0.95, 1.43	
Hysterectomy + oophorectomy	82,879	95	1.26	1.01, 1.56	
Missing data		314			
PHT status[c]					
Never use	1,797,159	657	1.00	Referent	
Ever use	1,001,200	427	1.03	0.91, 1.18	
Missing data		87			
Duration of PHT, years					0.11
0 (never use)	1,797,159	657	1.00	Referent	
0.10–1.25 (T1)	330,183	115	0.98	0.80, 1.20	
1.26–4.00 (T2)	345,001	123	1.01	0.83, 1.24	
>4.00 (T3)	317,805	136	1.00	0.82, 1.22	
Missing data		53			
Type of PHT[c]					
Never use	1,797,159	657	1.00	Referent	
Estrogen alone	428,936	137	0.95	0.78, 1.17	
Progestin alone	11,837	5	1.27	0.51, 3.12	
Estrogen + progestin	290,446	131	1.13	0.92, 1.38	
Missing data		241			

Abbreviations: CI, confidence interval; HR, hazard ratio; PHT, postmenopausal hormone therapy; T, tertile.
[a] Numbers may not sum to totals because of missing values.
[b] All models stratified the data by center and age and adjusted the results for educational level.
[c] Among peri- and postmenopausal women (including women with surgical menopause) (n = 1,171 cases).

factors and exogenous hormone use and B-cell NHL, except for a moderately increased risk among women who at baseline reported having had a surgical menopause as compared with women who did not. Hysterectomy alone was not associated with B-cell NHL risk, while women with hysterectomy plus oophorectomy (especially bilateral oophorectomy) showed a higher risk of B-cell NHL.

The subject of the role of reproductive factors in lymphomagenesis has been controversial. Evidence for a role of hormonal factors in NHL etiology comes mainly from observational data summarized in a systematic review of the literature (10) and randomized data from the Women's Health Initiative Clinical Trial (15). In summary, regarding observational data, 7 cohort studies (16–25), 13 case-control studies (26–39), and pooled analysis of 2 consortia of case-control studies (40–42) found associations between reproductive factors or hormone use and incident lymphoma. Several studies lacked detailed data on hormonal exposures and used heterogeneous definitions of lymphoma and hormonal exposures that hampered the performance of a meta-analysis (10). The present analysis was based on detailed hormonal assessments, and it was the third largest individual study in terms of number of cases after 2 registry-based studies carried out in Sweden and Denmark, which assessed pregnancy variables and included 1,744 and 1,573 cases, respectively (20, 28).

Our finding of no association with parity is consistent with findings from the pooled analyses of the International Lymphoma Epidemiology Consortium (InterLymph), which included 3,816 cases and 5,151 controls from 18 studies (40). We observed null results for oral contraception, in accordance with previous studies (16, 23, 24). Postmenopausal hormone therapy has yielded contradictory findings. The protective role of hormone therapy observed in case-control studies (31–33, 36, 39, 42) has not been replicated in cohort studies (16, 19, 21, 24, 25). Additionally, in a systematic review of the literature, we concluded that the association between NHL and postmenopausal hormone therapy probably depends on the formulation used and oophorectomy status, which have been rarely assessed (10). When these data were available, associations were derived from unopposed estrogen use, rather than use of estrogen + progestin, although there were still inconsistencies. However, in the present cohort analyses, we did not find associations between B-cell NHL and unopposed estrogen use or combined therapy. Randomized data can help in disentangling the role of hormone use in lymphoma risk and help to avoid biases commonly observed in observational studies (43). In the Women's Health Initiative Clinical Trial, conjugated equine estrogens plus medroxyprogesterone acetate or conjugated equine estrogens alone were tested against placebo, and incidence rates of NHL were calculated by treatment group (15). During the 13 years of follow-up, 27,229 women were randomized to treatments, and 383 incident NHL cases were identified. In that study, incidence of NHL was similar in the treatment and placebo groups. Together with our data and other cohort data, this suggests that hormone therapy is not associated with NHL.

We observed that women with surgical menopause, particularly women with hysterectomy and bilateral oophorectomy, showed a significantly higher risk of B-cell NHL. The ovaries secrete sex hormones, and several studies have suggested that women who undergo bilateral oophorectomy have reduced serum concentrations of androgens, rather than estrogens, compared with postmenopausal women with intact ovaries (44, 45). However, steroid metabolism is very complex, and in vitro data suggest that androgens could modulate NHL risk in either direction (46, 47). Epidemiologic literature on the role of oophorectomy in NHL risk or its subtypes is scarce. In the California Teachers Study cohort, Lu et al. (25) observed that women with bilateral oophorectomy had a significant 37% increased risk of NHL compared with women with natural menopause, in accordance with our results. In a case-control study evaluating risk factors for multiple myeloma, surgical menopause by hysterectomy with bilateral oophorectomy was statistically significantly associated with an 85% increased risk of multiple myeloma (38). However, Lee et al. (31) found null results for NHL in a population-based case-control study, although women with oophorectomy and hysterectomy alone (without oophorectomy) were analyzed together and relevant misclassification of the surgical menopause may occurred. Considering our results and the 2 previous positive findings (25, 38), further assessment of bilateral oophorectomy is therefore warranted. However, the association was based on a small number of subjects, and the estimate for unilateral oophorectomy was below 1, which hampered the biological interpretation of this exposure. Therefore, this result could simply reflect type 1 error, given the large number of factors evaluated in the present analyses. Pooled analyses of cohort studies may provide the statistical power needed to corroborate or rule out these associations.

Our study was based on a large data set with a prospective design and detailed information on reproductive factors and exogenous hormone use, including formulations. In spite our relatively large sample size, associations for less common exposures among specific lymphoma subtypes relied on small numbers of cases. We had the ability to control for a variety of potential confounders, including education and body mass index, which biased previous studies of hormone therapy and disease because of confounding and a healthy user effect (43). However, these variables may imprecisely measure complex factors such as socioeconomic factors or adiposity, and therefore residual confounding cannot completely be ruled out. A concern in this study is that menstrual and reproductive variables were based on self-reported data. However, the reliability of responses to questions on reproductive history, including self-reported oophorectomy and use of hormones, has been shown to be very high (48). In addition, information on hormone therapy was not periodically updated; therefore, we could not evaluate incident use. Importantly, the reported association between B-cell NHL and surgical menopause may have been due to chance, because we performed multiple comparisons.

In conclusion, our prospective analysis does not support a strong role for reproductive factors or exogenous hormones in lymphomagenesis.

ACKNOWLEDGMENTS

Author affiliations: Unit of Molecular Epidemiology and Genetics in Infections and Cancer, Cancer Epidemiology Research Programme, Bellvitge Biomedical Research

Institute, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain (Laura Costas, Yolanda Benavente, Delphine Casabonne); Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Bellvitge Biomedical Research Institute, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain (Leila Lujan-Barroso, Antonio Agudo); Department of Nursing of Public Health, Mental Health and Maternity and Child Health, School of Nursing, Universitat de Barcelona, Barcelona, Spain (Leila Lujan-Barroso); Consortium for Biomedical Research in Epidemiology and Public Health, Madrid, Spain (Yolanda Benavente, Pilar Amiano, Eva Ardanaz, José María Huerta, Delphine Casabonne, Maria-Jose Sánchez); Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, Medical Sciences Division, University of Oxford, Oxford, United Kingdom (Naomi E. Allen); Public Health Division of Gipuzkoa, BioDonostia Health Research Institute, San Sebastian, Spain (Pilar Amiano); Navarra Public Health Institute, Pamplona, Spain (Eva Ardanaz); Unit of Hematology-Oncology, Centre Hospitalier de Versailles, Le Chesnay, France (Caroline Besson); Centre de Recherche en Epidémiologie et Santé des Populations, Faculté de Médecine–Université Paris-Sud and Faculté de Médecine–Université de Versailles Saint-Quentin-en-Yvelines, Institut National de la Santé et de la Recherche Médicale, Université Paris-Saclay, Villejuif, France (Caroline Besson, Iris Cervenka, Agnès Fournier); Institut Gustave Roussy, Villejuif, France (Caroline Besson, Iris Cervenka, Agnès Fournier); German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany (Heiner Boeing); Department of Determinants of Chronic Diseases, National Institute for Public Health and the Environment, Bilthoven, the Netherlands (Bas Bueno-de-Mesquita); Department of Gastroenterology and Hepatology, University Medical Centre Utrecht, Utrecht, the Netherlands (Bas Bueno-de-Mesquita); Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, St. Mary's Campus, London, United Kingdom (Bas Bueno-de-Mesquita); Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia (Bas Bueno-de-Mesquita); Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany (Renée T. Fortner, Rudolf Kaaks); International Agency for Research on Cancer, Lyon, France (Marc Gunter, Neil Murphy); Department of Radiation Sciences, Oncology, Faculty of Medicine, Umeå University, Umeå, Sweden (Sophia Harlid, Beatrice Melin); Department of Epidemiology, Murcia Regional Health Council, Instituto Murciano de Investigación Biosanitaria-Arrixaca, Murcia, Spain (José María Huerta); Division of Oncology and Pathology, Department of Clinical Sciences, Faculty of Medicine, Lund University, Lund, Sweden (Mats Jerkeman, Karin Jirström); Hellenic Health Foundation, Athens, Greece (Anna Karakatsani, Anastasia Kotanidou, Antonia Trichopoulou); Department of Pulmonary Medicine, School of Medicine, National and Kapodistrian University of Athens, Attikon University General Hospital, Haidari, Greece (Anna Karakatsani); Department of Public Health and Primary Care, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom (Kay-Tee Khaw); Department of Critical Care Medicine and Pulmonary Services, University of Athens Medical School, Evangelismos Hospital, Athens, Greece (Anastasia Kotanidou); Department of Critical Care Medicine and Pulmonary Services, University of Athens Medical School, Evangelismos Hospital, Athens, Greece (Anastasia Kotanidou); Department of Community Medicine, The Arctic University of Norway, Tromsø, Norway (Eiliv Lund, Elisabete Weiderpass); Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network, Florence, Italy (Giovanna Masala); Dipartimento di Medicina Clinica e Chirugia, Federico II University, Naples, Italy (Amalia Mattiello); Public Health Directorate, Asturias, Spain (Virginia Menéndez); Center for Chronic Immunodeficiency at the Center for Translational Cell Research, University of Freiburg Medical Center, Freiburg, Germany (Alexandra Nieters); Department of Public Health, Faculty of Health, Aarhus University, Aarhus, Denmark (Kim Overvad); Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom (Elio Riboli); Unit of Cancer Epidemiology, Città della Salute e della Scienza University-Hospital and Center for Cancer Prevention, Turin, Italy (Carlotta Sacerdote); Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria de Granada, Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain (Maria-Jose Sánchez); Cancer Epidemiology Unit, Nuffield Department of Population Health, Medical Sciences Division, University of Oxford, Oxford, United Kingdom (Julie A. Schmidt); Epidemiology and Prevention Unit, Fondazione IRCCS-Istituto Nazionale dei Tumori, Milano, Italy (Sabina Sieri); Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark (Anne Tjønneland); Cancer Registry and Histopathology Department, Maria Paternò Arezzo Hospital, Ragusa, Italy (Rosario Tumino); Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands (Roel Vermeulen); Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway (Elisabete Weiderpass); Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (Elisabete Weiderpass); Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland (Elisabete Weiderpass); Faculty of Medicine, University of Helsinki, Helsinki, Finland (Elisabete Weiderpass); and PATH, Seattle, Washington (Silvia de Sanjosé).

Coordination of the European Prospective Investigation Into Cancer and Nutrition (EPIC) is financially supported by the European Commission's Health and Consumer Protection Directorate General and the International Agency for Research on Cancer. The national cohorts are supported by the following organizations/institutions: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, and Institut National de la Santé et de la Recherche Médicale (France); German Cancer Aid, German Cancer Research Center, and Federal Ministry of Education and Research

(Germany); Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports, Netherlands Comprehensive Cancer Center, Zorg Onderzoek Nederland Medische Wetenschappen, World Cancer Research Fund, Dutch Cancer Society, and Statistics Netherlands (Netherlands); Regional Governments of Andalucía, Asturias, Basque Country, Murcia, and Navarra and Catalan Institute of Oncology (Spain); Swedish Cancer Society, Swedish Research Council, and County Councils of Skåne and Västerbotten (Sweden); and Cancer Research UK (grant 14136 to EPIC-Norfolk; grants C570/A16491 and C8221/A19170 to EPIC-Oxford) and Medical Research Council (grant 1000143 to EPIC-Norfolk and grant MR/M012190/1 to EPIC-Oxford) (United Kingdom).

Conflict of interest: none declared.

REFERENCES

1. Smith A, Roman E, Howell D, et al. The Haematological Malignancy Research Network (HMRN): a new information strategy for population based epidemiology and health service research. *Br J Haematol*. 2010;148(5):739–753.
2. Cutolo M, Brizzolara R, Atzeni F, et al. The immunomodulatory effects of estrogens: clinical relevance in immune-mediated rheumatic diseases. *Ann N Y Acad Sci*. 2010;1193:36–42.
3. Zen M, Ghirardello A, Iaccarino L, et al. Hormones, immune response, and pregnancy in healthy women and SLE patients. *Swiss Med Wkly*. 2010;140(13-14):187–201.
4. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update*. 2005;11(4):411–423.
5. Ladikou EE, Kassi E. The emerging role of estrogen in B cell malignancies. *Leuk Lymphoma*. 2017;58(3):528–539.
6. Yakimchuk K, Norin S, Kimby E, et al. Up-regulated estrogen receptor β2 in chronic lymphocytic leukemia. *Leuk Lymphoma*. 2012;53(1):139–144.
7. Shim GJ, Gherman D, Kim HJ, et al. Differential expression of oestrogen receptors in human secondary lymphoid tissues. *J Pathol*. 2006;208(3):408–414.
8. Yakimchuk K, Iravani M, Hasni MS, et al. Effect of ligand-activated estrogen receptor β on lymphoma growth in vitro and in vivo. *Leukemia*. 2011;25(7):1103–1110.
9. Shim GJ, Wang L, Andersson S, et al. Disruption of the estrogen receptor beta gene in mice causes myeloproliferative disease resembling chronic myeloid leukemia with lymphoid blast crisis. *Proc Natl Acad Sci U S A*. 2003;100(11):6694–6699.
10. Costas L, de Sanjosé S, Infante-Rivard C. Reproductive factors and non-Hodgkin lymphoma: a systematic review. *Crit Rev Oncol Hematol*. 2014;92(3):181–193.
11. Morton LM, Slager SL, Cerhan JR, et al. Etiologic heterogeneity among non-Hodgkin lymphoma subtypes: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. *J Natl Cancer Inst Monogr*. 2014;2014(48):130–144.
12. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002;5(6B):1113–1124.
13. Swerdlow SH; International Agency for Research on Cancer, World Health Organization. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: International Agency for Research on Cancer; 2008.
14. Wang M, Spiegelman D, Kuchiba A, et al. Statistical methods for studying disease subtype heterogeneity. *Stat Med*. 2016;35(5):782–800.
15. Kato I, Chlebowski RT, Hou L, et al. Menopausal estrogen therapy and non-Hodgkin's lymphoma: a *post-hoc* analysis of Women's Health Initiative randomized clinical trial. *Int J Cancer*. 2016;138(3):604–611.
16. Morton LM, Wang SS, Richesson DA, et al. Reproductive factors, exogenous hormone use and risk of lymphoid neoplasms among women in the National Institutes of Health-AARP Diet and Health Study cohort. *Int J Cancer*. 2009;124(11):2737–2743.
17. Prescott J, Lu Y, Chang ET, et al. Reproductive factors and non-Hodgkin lymphoma risk in the California Teachers Study. *PLoS One*. 2009;4(12):e8135.
18. Cerhan JR, Habermann TM, Vachon CM, et al. Menstrual and reproductive factors and risk of non-Hodgkin lymphoma: the Iowa Women's Health Study (United States). *Cancer Causes Control*. 2002;13(2):131–136.
19. Teras LR, Patel AV, Hildebrand JS, et al. Postmenopausal unopposed estrogen and estrogen plus progestin use and risk of non-Hodgkin lymphoma in the American Cancer Society Cancer Prevention Study-II cohort. *Leuk Lymphoma*. 2013;54(4):720–725.
20. Frisch M, Pedersen BV, Wohlfahrt J, et al. Reproductive patterns and non-Hodgkin lymphoma risk in Danish women and men. *Eur J Epidemiol*. 2006;21(9):673–679.
21. Nørgaard M, Poulsen AH, Pedersen L, et al. Use of postmenopausal hormone replacement therapy and risk of non-Hodgkin's lymphoma: a Danish population-based cohort study. *Br J Cancer*. 2006;94(9):1339–1341.
22. Kvåle G, Heuch I, Nilssen S. Parity in relation to mortality and cancer incidence: a prospective study of Norwegian women. *Int J Epidemiol*. 1994;23(4):691–699.
23. Michels KA, Brinton LA, Pfeiffer RM, et al. Oral contraceptive use and risks of cancer in the NIH-AARP Diet and Health Study. *Am J Epidemiol*. 2018;187(8):1630–1641.
24. Cerhan JR, Wallace RB, Folsom AR, et al. Medical history risk factors for non-Hodgkin's lymphoma in older women. *J Natl Cancer Inst*. 1997;89(4):314–318.
25. Lu Y, Wang SS, Sullivan-Halley J, et al. Oral contraceptives, menopausal hormone therapy use and risk of B-cell non-Hodgkin lymphoma in the California Teachers Study. *Int J Cancer*. 2011;129(4):974–982.
26. Costas L, Casabonne D, Benavente Y, et al. Reproductive factors and lymphoid neoplasms in Europe: findings from the EpiLymph case-control study. *Cancer Causes Control*. 2012;23(1):195–206.
27. Miller AB, Barclay THC, Choi NW, et al. A study of cancer, parity and age at first pregnancy. *J Chronic Dis*. 1980;33(10):595–605.
28. Adami HO, Tsaih S, Lambe M, et al. Pregnancy and risk of non-Hodgkin's lymphoma: a prospective study. *Int J Cancer*. 1997;70(2):155–158.
29. Bernstein L, Ross RK. Prior medication use and health history as risk factors for non-Hodgkin's lymphoma: preliminary results from a case-control study in Los Angeles County. *Cancer Res*. 1992;52(19 suppl):5510s–5515s.
30. Zhang Y, Holford TR, Leaderer B, et al. Menstrual and reproductive factors and risk of non-Hodgkin's lymphoma among Connecticut women. *Am J Epidemiol*. 2004;160(8):766–773.

31. Lee JS, Bracci PM, Holly EA. Non-Hodgkin lymphoma in women: reproductive factors and exogenous hormone use. *Am J Epidemiol*. 2008;168(3):278–288.
32. Nelson RA, Levine AM, Bernstein L. Reproductive factors and risk of intermediate- or high-grade B-cell non-Hodgkin's lymphoma in women. *J Clin Oncol*. 2001;19(5):1381–1387.
33. Beiderbeck AB, Holly EA, Sturkenboom MC, et al. No increased risk of non-Hodgkin's lymphoma with steroids, estrogens and psychotropics (Netherlands). *Cancer Causes Control*. 2003;14(7):639–644.
34. La Vecchia C, Negri E, Franceschi S, et al. Long-term impact of reproductive factors on cancer risk. *Int J Cancer*. 1993; 53(2):215–219.
35. Olsson H, Olsson ML, Ranstam J. Late age at first full-term pregnancy as a risk factor for women with malignant lymphoma. *Neoplasma*. 1990;37(2):185–190.
36. Mildon KH, Ansell P, Roman E, et al. Reproductive factors, menopausal hormone therapy, and risk of non-Hodgkin, diffuse large B-cell and follicular lymphomas: a UK case-control study. *Cancer Causes Control*. 2010;21(12): 2079–2083.
37. Tavani A, Pregnolato A, La Vecchia C, et al. A case-control study of reproductive factors and risk of lymphomas and myelomas. *Leuk Res*. 1997;21(9):885–888.
38. Wang SS, Voutsinas J, Chang ET, et al. Anthropometric, behavioral, and female reproductive factors and risk of multiple myeloma: a pooled analysis. *Cancer Causes Control*. 2013;24(7):1279–1289.
39. Zhang Y, Holford TR, Leaderer B, et al. Prior medical conditions and medication use and risk of non-Hodgkin lymphoma in Connecticut United States women. *Cancer Causes Control*. 2004;15(4):419–428.
40. Kane EV, Roman E, Becker N, et al. Menstrual and reproductive factors, and hormonal contraception use: associations with non-Hodgkin lymphoma in a pooled analysis of InterLymph case-control studies. *Ann Oncol*. 2012;23(9): 2362–2374.
41. Costas L, Lambert BH, Birmann BM, et al. A pooled analysis of reproductive factors, exogenous hormone use, and risk of multiple myeloma among women in the International Multiple Myeloma Consortium. *Cancer Epidemiol Biomarkers Prev*. 2016;25(1):217–221.
42. Kane EV, Bernstein L, Bracci PM, et al. Postmenopausal hormone therapy and non-Hodgkin lymphoma: a pooled analysis of InterLymph case-control studies. *Ann Oncol*. 2013; 24(2):433–441.
43. Humphrey LL, Chan BK, Sox HC. Postmenopausal hormone replacement therapy and the primary prevention of cardiovascular disease. *Ann Intern Med*. 2002;137(4):273–284.
44. Davison SL, Bell R, Donath S, et al. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab*. 2005;90(7):3847–3853.
45. Fogle RH, Stanczyk FZ, Zhang X, et al. Ovarian androgen production in postmenopausal women. *J Clin Endocrinol Metab*. 2007;92(8):3040–3043.
46. Mostaghel EA, Martin PS, Mongovin S, et al. Androgen receptor expression in mantle cell lymphoma: potential novel therapeutic implications. *Exp Hematol*. 2017;49:34–38.e2.
47. Talaber G, Yakimchuk K, Guan J, et al. Inhibition of estrogen biosynthesis enhances lymphoma growth in mice. *Oncotarget*. 2016;7(15):20718–20727.
48. Lucas R, Azevedo A, Barros H. Self-reported data on reproductive variables were reliable among postmenopausal women. *J Clin Epidemiol*. 2008;61(9):945–950.

PAPER – IV

Menstrual factors, reproductive history, hormone use, and Urothelial carcinoma risk: A prospective study in the EPIC cohort

Lujan-Barroso L, Botteri E, Caini S, Ljungberg B, Roswall N, Tjønneland A, Bueno-de-Mesquita B, Gram IT, Rosario Tumino, Kiemeney LA, Liedberg F, Stocks T, Gunter M, Murphy N, Cervenka I, Fournier A, Kvaskoff M, Christel Häggström, Overvad K, Lund E, Waaseth M, Fortner RT, Kühn T, Menéndez V, Sánchez MJ, Santiuste C, Perez-Cornago A, Zamora-Ros R, Cross AJ, Trichopoulou A, Karakatsani A, Peppa E, Domenico Palli, Krogh V, Sciannameo V, Mattiello A, Panico S, van Gils CH, N. Onland-Moret C, Barricarte A, Amiano P, Khaw KT, Heiner Boeing, Weiderpass E, Duell EJ

Under review in the British Journal of Cancer

Menstrual factors, reproductive history, hormone use, and Urothelial carcinoma risk: A prospective study in the EPIC cohort

Running title: Reproductive factors and Urothelial carcinoma

Leila Lujan-Barroso 1,2,3, Edoardo Botteri 4, 5, Saverio Caini 6, Börje Ljungberg 7, Nina Roswall 8, Anne Tjønneland 8,9 , Bas Bueno-de-Mesquita 10, 11, 12, 13, Inger T Gram 14, Rosario Tumino 15, Lambertus A Kiemeney 16, Fredrik Liedberg 17, Tanja Stocks 18, Marc Gunter 19, Neil Murphy 19, Iris Cervenka 20, Agnès Fournier 20, Marina Kvaskoff 20, Christel Häggström 21, 22, Kim Overvad 23, Eiliv Lund 14, Marit Waaseth 24, Renée Turzanski Fortner 25, Tilman Kühn 25, Virginia Menéndez 26, Maria-Jose Sánchez 27,28, Carmen Santiuste 28,29, Aurora Perez-Cornago 30, Raul Zamora-Ros 1,2, Amanda J Cross 31, Antonia Trichopoulou 32, Anna Karakatsani 32,33, Eleni Peppa 32, Domenico Palli 6, Vittorio Krogh 34, Veronica Sciannameo 35, Amalia Mattiello 36, Salvatore Panico 36, Carla H. van Gils 37, N. Charlotte Onland-Moret 37, Aurelio Barricarte 28, 38, 39, Pilar Amiano 28, 40, Kay-Tee Khaw 41, Heiner Boeing 42, Elisabete Weiderpass[*] 19, Eric J Duell[*] 43, 44.

1. Unit of Nutrition and Cancer. Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.
2. Bellvitge Biomedical Research Institute – IDIBELL, Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.
3. Department of Nursing of Public Health, Mental Health and Maternity and Child Health School of Nursing Universitat de Barcelona, Carrer de la Feixa Llarga s/n, 08907, L'Hospitalet de Llobregat, Barcelona, Spain
4. Cancer Registry of Norway, Oslo University Hospital, Ullernchausseen 64, 0379, Oslo, Norway.
5. Norwegian National Advisory Unit for Women's Health, Women's Clinic, Oslo University Hospital, Sognsvannsveien 20, 0372, Oslo, Norway.
6. Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Via Cosimo il Vecchio 2, 50139, Florence, Italy

7. Department of surgical and perioperative sciences, urology and andrology, Umeå University, 901 85, Umeå, Sweden.

8. Diet, Genes and Environment, Danish Cancer Society Research Center, Strandboulevarden 49, DK-2100, Copenhagen Copenhagen, Denmark.

9. Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Øster Farimagsgade 5, 1014 Copenhagen.

10. Former senior scientist, Dept. for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands.

11. Former associate professor, Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands.

12. Visiting professor, Dept. of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, St Mary's Campus, Norfolk Place, London, W2 1PG London, United Kingdom.

13. Academic Icon / visiting professor, Dept. of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Pantai Valley, 50603, Kuala Lumpur, Malaysia.

14. Department of Community Medicine, University of Tromsø , The Arctic University of Norway, N – 9037, Tromsø, Norway.

15. Cancer Registry and histopathology Department, "Civic -M.P. Arezzo" Hospital, ASP Ragusa, 97100, Ragusa, Italy.

16. Radboud university medical center, Radboud Institute for Health Sciences, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

17. Department of Urology Skåne University Hospital and Institution of Translational Medicine, Lund University, Jan Waldenströms gata 5, 205 02, Malmö, Sweden.

18. Department of Clinical Sciences Lund, Lund University, Barngatan 4, 222 42, Lund, Sweden.

19. International Agency for Research on Cancer / World Health Organization, 150 cours Albert Thomas 69372, Lyon CEDEX 08, France.

20. Inserm U1018, Centre for Research in Epidemiology and Population Health (CESP) "Health across Generations" Team, Gustave Roussy 114 rue Edouard Vaillant, F-94805, Villejuif, France.

21. Department of Biobank Research, Umeå University, SE-901 87, Umeå, Sweden.

22. Department of Surgical Sciences, Uppsala University, Akademiska sjukhuset entrence 70, 1 tr SE-751 85, Uppsala, Sweden.

23. Department of Public Health, Section for Epidemiology, Aarhus University, Bartholins Allé 2 DK-8000, Aarhus, Denmark.

24. Department of Pharmacy, University of Tromsø , The Arctic University of Norway, N – 9037, Tromsø, Norway.

25. Division of Cancer Epidemiology, German Cancer Research Center (DFKZ), Im Neuenheimer Feld 280, · 69120, Heidelberg, Germany.

26. Public Health Directorate, C/Ciriaco Miguel Virgil 9, 33006, Oviedo, Asturias, Spain

27. Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.GRANADA. Hospitales Universitarios de Granada/Universidad de Granada, Cuesta del Observatorio 4, 18080, Granada, Spain

28. CIBER de Epidemiología y Salud Pública (CIBERESP), Av. Monforte de Lemos 3-5, 28029 Madrid, Spain

29. Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Ronda de Levante 11, 30008, Murcia, Spain.

30. Cancer Epidemiology Unit, Nuffield Department of Population Health University of Oxford, OX3 7LF,Oxford, United Kingdom.

31. Faculty of Medicine, Imperial College London, Norfolk Place, London W2 1PG, London, UK.

32. Hellenic Health Foundation, Kaisareias 13 & Alexandroupoleos, GR-115 27, Athens, Greece.

33. 2nd Pulmonary Medicine Department, School of Medicine, National and Kapodistrian University of Athens, "ATTIKON" University Hospital, 12462, Haidari, Greece.

34. Epidemiology and Prevention Unit. Fondazione IRCCS Istituto Nazionale dei Tumouri, Via Venezian 1, 20133 Milano-Italy.

35. Unit of Epidemiology, Regional Health Service ASL TO3, 10095 Grugliasco (Turin), Italy.

36. Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Via Pansini 5, 80131, Naples, Italy.

37. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, P.O. Box 85500, 3508 GA Utrecht, The Netherlands.

38. Navarra Public Health Institute, C/Leyre 15, 31003, Pamplona, Spain.

39. Navarra Institute for Health Research (IdiSNA), C/Irunlarrea 3, 31008, Pamplona, Spain.

40. Ministry of Health of the Basque Government, Public Health Division of Gipuzkoa. Biodonostia Research Institute: Paseo Doctor Begiristain s/N, 20014 Donostia/Gipuzkoa, Gipuzkoa, Spain

41. Department of Public Health and Primary Care, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 0SP, United Kingdom.

42. German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Arthur-Scheunert-Allee 114 – 116, 14558 Nuthetal, Germany.

43. Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program, Catalan Institute of Oncology (ICO), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.

44. ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.

*These authors contributed equally to this work

Corresponding author:

Leila Luján-Barroso, MSC

Unit of Nutrition and Cancer

Cancer Epidemiology Research Program

Cantaln Institute of Oncology (ICO-IDIBELL)

Avda. Gran Via 199-203

08908 L'Hospitalet de Llobregat, Barcelona, Spain

Tel: +34 93 260 7401

Fax: +34 93 260 7787

email: llujan@iconcologia.net

ORCID: 0000-0001-6224-1764

Word count: Abstract 200 words; Text 3,019 words; 3 tables; 2 supplementary tables; 31 references

Abstract:

Background

Urothelial carcinoma (UC) is the predominant (95%) bladder cancer cell type in industrialised nations. Animal and human studies suggest that hormonal factors may influence UC risk**.**

Methods

We used an analytic cohort of 333 919 women from the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC). Associations between exposure variables and incident UC risk were evaluated using Cox proportional hazards models. All models were stratified by age at recruitment and study centre, and adjusted for smoking status and intensity, fruit and vegetable intakes.

Results

During a mean of 15 years of follow-up, 529 women developed UC. A final mutually-adjusted model including number of full-term pregnancies (FTP), menopausal status, and menopausal hormone therapy (MHT) showed an inverse association between number of FTP and UC ($HR_{\geq 5 vs 1}$=0.48, 0.25-0.90; *P*-trend in parous women=0.010). MHT-use (compared to non-use) was positively associated with UC (HR=1.27, 1.03-1.57). No modification of HRs by smoking status was observed. Sensitivity analysis in never-smokers showed similar HRs patterns for number of FTP and no association between MHT-use and UC.

Conclusion

We observed that increasing number of FTP may reduce UC risk and limited evidence of the role of MHT-use in UC. More detailed studies on parity are needed to understand any effect of perinatal hormone changes in urothelial cells.

Key words: Bladder cancer; menopausal hormone therapy; menstrual and reproductive factors; parity; urothelial carcinoma.

Introduction:

Bladder cancer is the 12th most common cancer in the world, accounting for 4.8% and 1.5% of incident cancers in men and women, respectively(1). In 2018, the estimated male:female sex ratio in Europe was 4.7 to 1(1). The predominant bladder cancer cell type is urothelial carcinoma (UC), accounting for 95% of all cases in industrialise nations(2). Between 50-64% of UC cases in men and 20-50% in women are attributable to tobacco use; and the risk increases with both intensity and duration of smoking(3). Other established risk factors for UC include occupational exposure to aromatic amines and dyes, ingestion of inorganic arsenic via drinking water, a positive family history, and constitutional variants in at least a dozen genes(2,4).

Sex differences in UC incidence may be explained to a large extent by sex differences in the prevalence and intensity of exposure to known risk factors(2). However, several studies suggest that female hormones may have a beneficial effect on UC risk. An experimental animal study that examined effect of the hormones on oncogenesis in male rat bladders suggested that incidence of bladder cancer was higher in the group with testosterone supplementation than in the group with oestrogen supplementation (5). Moreover, castration of male mice and pregnancy and/or lactation in female mice can decrease the growth of bladder cancer(6). Previous epidemiological studies have reported a reduced risk of UC in parous women compared to nulliparous women(7–10); and an increased risk in postmenopausal women, particularly those with an earlier age at menopause(9,11,12). In general, no associations between age at menarche, the use of oral contraceptives (OC), age at first full-term pregnancy, and breastfeeding and UC

have been observed(7–17). A meta-analysis by MHT formulation(9), based on four studies, showed a possible reduction in risk of UC in women who used oestrogen plus progestin MHT compared to never users of MHT. Nevertheless, in the Women's Health Initiative (WHI), which included a clinical trial component and an observational study component, no such association was observed(16).

The aim of the present study was to assess the associations between menstrual factors, reproductive history, use of exogenous hormones, and the risk of developing UC, both overall and by tumour grade and by tumour aggressiveness, and accounting for smoking status, within a prospective cohort study of European women.

Methods:

Study design and population

The European Prospective Investigation into Cancer and Nutrition Cohort (EPIC) is an ongoing multicentre cohort study that recruited participants from 23 centres located in ten European countries. At recruitment (baseline), information on diet, lifestyle, and anthropometric measurements was collected. Lifestyle questionnaires included questions on education, occupation, medical history, lifetime history of consumption of tobacco, alcoholic beverages, and physical activity. Questionnaires specific to women were used to collect information on menstrual factors, reproductive history, and use of exogenous hormones. Details on the study design have been described previously(18). A total of 521 324 participants were recruited between 1992 and 2000.

Participants with prevalent cancers, except non-melanoma skin cancer, or participants with missing follow-up information were excluded (n=29 332). Only women were eligible for the present analysis (n=343 985). Women with incomplete information on dietary intake or lifestyle or who had extreme or implausible caloric intake (top or

bottom 1% of the ratio of energy intake to estimated energy required(19)) were excluded (n=10 066). After these exclusions, the present analysis included 333 919 women.

Exposure of interests

Self-reported menstrual factors, and exogenous hormone use included: age at menarche (<12, 12, 13, 14, >14 years), history (yes/no) and duration of OC use (non-user, >0-≤1, >1-5, >5-10 years), menopausal status at baseline (premenopausal: ≥9 cycles over the past 12 months, perimenopausal: <9 cycles, natural menopause in case of no menses, and surgical menopause in case of bilateral oophorectomy), age at natural menopause (surgical menopause were excluded, ≤46, 47-49, 50-52, ≥53 years) , age at any menopause (surgical and natural, ≤46, 47-49, 50-52, ≥53 years) , MHT-use (yes/no) and duration (non-user, >0-≤1.25, >1.25-4, >4 years), type of MHT (oestrogen alone, progestin alone, or oestrogen plus progestin), oophorectomy (yes/no), hysterectomy (yes/no), and calculated cumulative duration of menstrual cycling. Cumulative duration of menstrual cycling (in years) was calculated as follows: for postmenopausal women, it was the difference between the age at menopause and the age at menarche minus the total time pregnant (number of FTP x 9 months). For pre- and perimenopausal women, cumulative duration of menstrual cycling was the difference between age at recruitment and age at menarche minus the total time pregnant. Total time taking OCs was subtracted from cumulative duration of menstrual cycling(20) for pre-, peri-, and postmenopausal women.

Self-reported reproductive history included: parity (yes/no), number of full-term pregnancies (FTP, including livebirths and stillbirths; 0, 1, 2, 3, 4, ≥5), age at first FTP (in parous women; ≤20, 21-13, 24-25, 26-30, ≥30 years), number of induced (never pregnant, 0, 1, ≥2) and spontaneous abortions (never pregnant, 0, 1, ≥2), breastfeeding

(in parous women; yes/no), and duration of breastfeeding (in parous women who breastfeed; 0>-≤3, >3-12, >12 months).

Endpoint assessments

Incident bladder cancers were identified through population registries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden, and United Kingdom) and active follow-up, including use of health insurance records, hospital registries, and direct contacts with participants or next-of-kin (France, Germany, and Greece). For these analyses, the follow-up for UC was completed between December 2011 and December 2013, depending on the centre.

Bladder cancers were defined by ICD-O-3, including first invasive cancer (coded C67 based) and UC (morphology codes 812*–813*)(21). Only incident UC was included in the present analyses; since it represents 95% of all bladder cancers. Definitions of UC subtype classifications are heterogeneous in the literature. In previous EPIC studies, UC was classified by pathology reports as aggressive (pT1 and higher or carcinoma *in situ* (CIS) or World Health Organization (WHO) Grade 3), and non-aggressive (pTa Grade 1 and 2)(21). We also analysed UC by tumour grade (using WHO-defined Grades 2 and 3 as "high-grade" and Grade 1 as "low-grade")(22).

Statistical analysis

To evaluate associations between hormonal factors and UC risk, Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CI). Ordinal variables were scored and trend tests were calculated on these scores, "unknown" category was excluded for trend test calculation. Age was used as the time scale, with age at recruitment as the entry time, and age at the date of UC or the end of follow-up (whichever came first) as the exit time. Additional models were performed to describe the risk of UC by tumour aggressiveness and tumour grade. All

models were stratified by age at recruitment (1 year-categories) and study centre, and adjusted for smoking status and intensity at baseline (never-smokers, current smokers ≤15 cigarettes/day, current smokers >15 cigarettes/day, ex-smokers ≤10 years, ex-smokers >10 years, current: pipe/cigar/occasional cigarette smokers, current/former: missing intensity, and unknown), and fruit (g/d) and vegetable (g/d) intake(2). Physical activity and body mass index (BMI) were not included as adjustment covariates because they did not change effect estimates >10%. Other potential confounders were occupations at risk of potentially carcinogenic exposures. To adjust models for occupational risks a dichotomous score (yes/no) was defined, where it was coded as "yes" if the participant worked in occupations with exposure to heavy metals (present in foundries, in metal industries, and in occupation related to welding, turning and electroplating), aromatic amines (present in, e.g. dye production, textile and leather dying, and hairdressers), PAHs (polycyclic aromatic hydrocarbons; associated with refineries, asphalt work, the transport sector, and car repair stations), and environmental tobacco smoking (particularly elevated for workers in bars and restaurants), detailed information in Büchner *et al* (2009)(23). Nevertheless, occupation was ultimately not included in the multivariable-adjusted models because <7% of women worked in a potential high-risk job for UC, and adjusting for occupational exposure did not change any estimated HRs. To evaluate all identified factors in one model, mutually-adjusted models were evaluated. The proportional hazard assumption was checked using Schoenfeld residuals. Also, all the time-dependent variables (interactions of predictors and time) were included in the mutually-adjusted model and evaluated.

Modification of the HRs by tobacco use at baseline (never, former, and current) was evaluated using a likelihood ratio test (LRT). Joint effect variables (with a common

referent group) for tobacco with each variable included in the final model were also evaluated.

Sensitivity analyses were performed in never smokers to reduce the likelihood of residual confounding by smoking at baseline. Finally, to address possible changes in the reproductive history during the follow-up, a sensitivity analysis including only women with completed reproductive history (peri-/postmenopausal women at recruitment) was performed for the final model.

All statistical tests were two-sided and evaluated at α-level 0.05. All analyses were performed using SAS v. 9.4 (Cary, North Carolina, USA).

Results:

Descriptive statistics

After a median follow-up time of 15 years, 529 UC cases were identified including 146 non-aggressive tumours, 230 aggressive tumours, and 153 with unknown tumour aggressiveness; and among the 529 cases, there were 80 low-grade tumours, 233 high-grade tumours, and 216 with unknown tumour grade. The median age at recruitment was 51 years (y) (25^{th} and 75^{th} percentile (p25-p75): 45-58-y) for the whole cohort and 58-y (p25-p75: 52-63-y) for UC cases. The median age at diagnosis was 68-y (p25-p75: 62-74-y). Baseline characteristics of participants by country are presented in Supplemental Table 1.

Menstrual factors, and exogenous hormone use

Age at menarche, cumulative duration of menstrual cycling, history and duration of OC use, age at natural menopause, oophorectomy, and hysterectomy showed no association with UC risk (Table 1). Elevated and statistically significant HRs for UC were observed for postmenopausal status (natural or surgical) compared to premenopausal status ($HR_{naturalvspre}$: 1.88; 95%CI, 1.09-3.25; $HR_{surgicalvspre}$: 2.15; 95%CI, 1.10-4.20) (Table 1). MHT use in peri-/postmenopausal women (natural or surgical) was associated with overall UC independently of the duration of MHT use (Table 1). For the 67% (n=52,892, 82 cases) of women with information on formulation of MHT available, 25% (n=13,123, 32 cases) took oestrogen alone (HR: 1.43; 95%CI: 0.97-2.10). No association was observed for use of oestrogen plus progestin MHT formulations (HR: 1.08; 95%CI, 0.77- 1.51) (Table 1).

Reproductive factors

There was a statistically significant inverse association for number of FTP and UC risk (HR_{3vs1}: 0.70; 95%CI, 0.52-0.94; $HR_{\geq 5vs1FTP}$: 0.46; 95%CI, 0.25-0.88; *P*-trend in parous women only = 0.008). No statistically significant associations were observed for the other variables in Table 2.

Results on menstrual factors, reproductive factors, and exogenous hormone use by tumour aggressiveness and tumour grade are presented in supplemental table 2.

Mutually-adjusted Cox proportional hazards regression for UC

Models included number of FTP and menopausal status, where peri-/postmenopausal women were further classified by MHT history. Statistically significant inverse associations between number of FTP and UC risk were observed (HR_{3vs1}: 0.70; 95%CI,

0.52-0.94; $HR_{\geq 5 vs 1 FTP}$: 0.48; 95%CI, 0.25-0.90; *P*-trend in parous women only 0.010) (Table 3). Further, the HR for peri-/postmenopausal MHT-users compared to peri-/postmenopausal women never-users was 1.27 (95%CI, 1.03-1.57) (Table 3).

Modification of the HRs by tobacco

No evidence for modification of HRs for each factor and UC by cigarette smoking status was found (all likelihood ratio statistics *P*-value>0.05) with the exception of induced abortions (*P*-value=0.028). Different estimations of the HR of the number of induced abortions were observed by smoking status. While no association between number of induced abortions and the risk of UC was observed; HR for never smoking women with at least 2 induced abortions compare to 0 abortions was 2.52 (95%CI: 1.33- 4.78, *P*-trend = 0.012) (Supplemental table 3).

No modification of HRs in the mutually adjusted model by cigarette smoking status was observed. Nonetheless, the higher risk of MHT-use was only observed in peri-/postmenopausal women (natural or surgical) who were smokers at baseline (HR: 1.56; 95%CI: 1.10, 2.21) (Supplemental table 4). No statistically significant associations were observed when joint-effect variables for tobacco and FTP, and tobacco and menopausal status were evaluated.

Sensitivity analyses

In general, patterns of HRs did not change substantially when we restricted analyses to the subgroup of never smokers (Supplemental table 3 and Supplemental table 4), nor in the subgroup of participants who were peri-/postmenopausal at recruitment (data not shown).

Discussion:

The present analyses based on 529 women, showed evidence that women who had experienced more than one birth are at lower risk of developing UC compared to uniparous women; further, we observed evidence of an inverse trend between UC risk and number of births. Furthermore, in peri-/postmenopausal women, MHT-use may increase the risk of UC. No associations were observed for the remaining menstrual factors, reproductive history variables, or exogenous hormone use variables. Never smoking women who had two or more induced abortions were at higher risk of UC compared to women with no abortions.

Previous studies(9,10,16) and two meta-analyses(8,15) observed a reduced risk of UC in parous women, independent of the number of births(8,9,11,12,14–16). Nearly all these studies used “nulliparous” as the referent category(9,11,12,14,15). Nulliparous women likely represent a heterogeneous group that includes women with and women without fertility problems. In our study, “one birth” was used as a referent category, and we found a linear trend of decreasing UC risk with increasing number of FTP. This reduction in risk with increasing FTP was also observed in never-smokers. The observed trend in our study was similar to the trend reported by Weibull et al. (HR for ≥3 vs. 1 FTP: 0.76; 95%CI: 0.68-0.86)(10).

Women experience several hormonal changes during pregnancy, including an increase in oestrogen and progesterone levels(24). An animal study observed that these increased levels, particularly progesterone levels, may be related with changes in the bladder structure related to greater bladder capacity and compliance(25). Further, it has been shown that oestrogen receptors (ER) and progesterone receptors (PR), that mediate oestrogen and progesterone levels, are expressed in both normal and cancerous

urothelial cells(26,27). ERs have different roles in cancer biology, in general ER-α has been related with cell growth, while ER-β has been suggested to act as a suppressor of tumour growth, thus ER-α and ER-β may have opposing effects on cellular processes(28). It has been observed that ER-β is the dominant receptor expressed in urothelial carcinoma cells(6,26). Few studies have been done in relation to ERs and progesterone in urothelial carcinoma cells, but it has been suggested that progesterone suppress ER expression during pregnancy(29). Consequently, It can be hypothesized that these increased levels of oestrogen and progesterone may reduce UC risk in parous women(7–10,15,30).

Two previous studies have examined the association between induced abortions and the risk of UC (13,31). These two case-control studies did not observe that the number of induced abortion was associated with UC risk. Our results on never-smokers were based in a small number of cases, and in view of the large number of associations tested, the association in never-smokers between induced abortion and UC risk may be due to chance.

It has been hypothesized that earlier age at menopause increases UC risk due to lower levels of oestrogen after menopause(12). Earlier age at menopause (natural or surgical) was associated with an increased risk of UC in a meta-analysis(15), that included 4 case-control studies and 3 cohort studies. We observed no association between earlier age at menopause and UC, in agreement with other recent prospective cohort studies(8,9,16).

The higher UC risk we observed in peri-/postmenopausal MHT users, when compared to peri-/postmenopausal non-users, is inconsistent with previous studies which found no relation(8,15,16). Since no association was observed in never-smokers, and the overall MHT effect only remained significant in current-smokers, residual confounding from

tobacco smoking is a likely explanation for our MHT results. The WHI found no influence of the formulation of MHT on the risk of UC (results for oestrogen: n=136 cases; HR: 0.93; 95%CI: 0.74-1.17; results for oestrogen plus progestin: n=103 cases; HR: 1.05; 95%CI: 0.81-1.36)(16). A meta-analysis (based on 4 cohort studies) of MHT by formulation (oestrogen or oestrogen plus progestin) showed a 39% decreased UC risk in users of oestrogen plus progestin (n=84 cases; RR: 0.61; 95%CI: 0.47-0.78), and no effect for users of oestrogen alone (n=217 cases; RR: 1.03; 95%CI: 0.87-1.24)(9). Our results, based on smaller sample sizes (52 UC for oestrogen, and 30 UC for oestrogen plus progestin), were in agreement with those from the WHI.

Our study strengths include its prospective cohort design and a relatively large number of incident cases from 10 European countries, which allowed us to investigate associations by strata of smoking status. To our knowledge, this is the first study on menstrual factors, reproductive history, hormone use, and UC risk that includes information on tumour classification.

One potential weakness of our analysis is that information on reproductive history and hormone use was available only at cohort enrolment; however, we noted that 78.7% of the cases were postmenopausal at recruitment, so reproductive history was essentially complete for most participants. We performed sensitivity analyses restricted to postmenopausal women, whose reproductive exposures were unlikely to change. We observed similar results for the final mutually-adjusted model in the analysis restricted to postmenopausal women as we observed for all study participants. Thus, our results were unlikely to be affected by changes in reproductive history during the follow-up. Another potential weakness of our study was the large number of missing values in the MHT variables (duration and formulation). We observed almost the twice of missing MHT information in women diagnosed with UC who were current-smokers than those

who were never-smokers. Thus, it seems that current-smokers tended to omit their use of MHT and their risk of UC maybe was underestimated. Also, information on MHT was not periodically updated, and therefore, we could not evaluate risk in women who started using MHT or who modified their use after enrolment. Further, tumour grade and tumour aggressiveness had a large number of missing values which could bias HR estimates. Another weakness was that information on smoking habits was not periodically updated. Results from the sensitivity analyses in never smoking women showed that our results in general were not affected by residual confounding by smoking status. Finally, we could not consider occupational exposure in our analysis, as not all EPIC-centres collected such information. Further, occupational exposure was available for 32% (n=169) of UC cases; of which 10% (n=17) reported jobs considered at risk. Despite this, a sensitivity analysis was performed including occupational exposures in the final UC model and similar HR estimates for menopausal status, MHT-use, and number of full-term pregnancies were observed.

Conclusion:

Our results confirm the increasing benefit of each birth after the first on UC risk. Our results provided little support for the hypothesis that MHT-use lowers the risk of UC. Results from other large cohorts and consortia with a large sample of never-smokers, might help to clarify the evidence provided by this analysis. More studies on number of FTP are needed to elucidate the putative “protective” effects of parity. Further investigations of the role of perinatal hormonal changes and how these changes may affect to ER and PR levels and urothelial cells in the bladder are needed.

Additional Information:

Ethics approval and consent to participate: The EPIC study was performed in accordance with the Declaration of Helsinki. All participants signed an informed consent form, and each centre obtained approval from the local Ethics Committee.

Consent for publication: Not applicable.

Data availability: Dataset of the study can be found with the corresponding author.

Conflict of interest: The authors declare that they have no conflicts of interest.

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

Funding: None

Author's contribution

LLB, EB, SC, EW, and EJD analyzed and interpreted the data. LLB, EW, and EJD wrote the manuscript. BL, NR, AT, BBdM, ITG, RT, LAK, FL, TS, MG, NM, IC, AF,MK, CH, KO, EL, MW, RTF, TK, VM, MJS, CS, APC, RZR, AJC, AT, AK, EP, DP, VK, VS, AM, SP, CHvG, NCOM, AB, PA, KTK, HB, and EW collected the data and provided critical comments on the manuscript.

Acknowledgments:

We thank CERCA Program / Generalitat de Catalunya for institutional suport. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German

Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Compagnia di SanPaolo (Naples, Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Comprehensive Cancer Center The Netherlands (IKNL), Zorg Onderzoek Nederland Medische Wetenschappen (ZONMW), World Cancer Research Fund (WCRF), Dutch Cancer Society (KWF), Statistics Netherlands (The Netherlands), Health Research Fund (FIS) - Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology - ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (UK). Raul Zamora-Ros would like to thank the “Miguel Servet” program (CP15/00100) from the Institute of Health Carlos III and European Social Fund (ESF). For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

References:

1. Global Cancer Observatory [Internet]. [cited 2018 Oct 23]. Available from: http://gco.iarc.fr/

2. Malats N, Real FX. Epidemiology of bladder cancer. Hematol Oncol Clin North Am. 2015 Apr;29(2):177–89, vii.

3. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011 Aug;306(7):737–45.

4. Bladder cancer statistics | World Cancer Research Fund International [Internet]. [cited 2017 Apr 11]. Available from: http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/bladder-cancer-statistics

5. Tanahashi NK, Suzawa N, Azuma C. Effects of sex hormones on oncogenesis in rat urinary bladder by N-butyl-N-(4-hydroxybutyl)-nitrosamine. Int J Clin Pharmacol Biopharm. 1977 Mar;15(3):101–5.

6. Johnson AM, O'Connell MJ, Messing EM, Reeder JE. Decreased bladder cancer growth in parous mice. Urology. 2008 Sep;72(3):470–3.

7. Huang A-T, Kogevinas M, Silverman DT, Malats N, Rothman N, Tardon A, et al. Bladder cancer and reproductive factors among women in Spain. Cancer Causes Control. 2009 Dec;20(10):1907–13.

8. Davis-Dao CA, Henderson KD, Sullivan-Halley J, Ma H, West D, Xiang Y-B, et al. Lower risk in parous women suggests that hormonal factors are important in bladder cancer etiology. Cancer Epidemiol Biomarkers Prev. 2011 Jun;20(6):1156–70.

9. Daugherty SE, Lacey JV, Pfeiffer RM, Park Y, Hoover RN, Silverman DT. Reproductive factors and menopausal hormone therapy and bladder cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2013 Jul;133(2):462–72.

10. Weibull CE, Eloranta S, Altman D, Johansson ALV, Lambe M. Childbearing and the risk of bladder cancer: a nationwide population-based cohort study. Eur Urol. 2013 Apr;63(4):733–8.

11. McGrath M, Michaud DS, De Vivo I. Hormonal and reproductive factors and the risk of bladder cancer in women. Am J Epidemiol. 2006 Feb;163(3):236–44.

12. Prizment AE, Anderson KE, Harlow BL, Folsom AR. Reproductive risk factors for incident bladder cancer: Iowa Women's Health Study. Int J Cancer. 2007 Mar;120(5):1093–8.

13. Pelucchi C, La Vecchia C, Negri E, Dal Maso L, Franceschi S. Smoking and other risk factors for bladder cancer in women. Prev Med. 2002 Aug;35(2):114–20.

14. Cantwell MM, Lacey JV, Schairer C, Schatzkin A, Michaud DS. Reproductive factors, exogenous hormone use and bladder cancer risk in a prospective study. Int J Cancer. 2006 Nov;119(10):2398–401.

15. Dietrich K, Demidenko E, Schned A, Zens MS, Heaney J, Karagas MR. Parity, early menopause and the incidence of bladder cancer in women: a case-control study and meta-analysis. Eur J Cancer. 2011 Mar;47(4):592–9.

16. Kabat GC, Kim MY, Luo J, Hou L, Cetnar J, Wactawski-Wende J, et al. Menstrual and reproductive factors and exogenous hormone use and risk of transitional cell bladder cancer in postmenopausal women. Eur J Cancer Prev. 2013 Sep;22(5):409–16.

17. Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. Int J Cancer. 2003 Jun;105(3):408–12.

18. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002 Dec;5(6B):1113–24.

19. Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr. 2002 Dec;5(6B):1329–45.

20. al DE et. Menstrual and reproductive factors, exogenous hormone use, and gastric cancer risk in a cohort of women from the European Prospective Investigation... - PubMed - NCBI [Internet]. [cited 2018 Jan 9]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/?term=duell+gastric+cancer+hormones

21. Roswall N, Freisling H, Bueno-de-Mesquita HB, Ros M, Christensen J, Overvad K, et al. Anthropometric measures and bladder cancer risk: a prospective study in the EPIC cohort. Int J Cancer. 2014 Dec;135(12):2918–29.

22. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, et al. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016." Eur Urol Focus. 2018 Jan 20;5(3):457–66.

23. Büchner FL, Bueno-de-Mesquita HB, Ros MM, Kampman E, Egevad L, Overvad K, et al. Consumption of vegetables and fruit and the risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2009 Dec 1;125(11):2643–51.

24. Modugno F, Laskey R, Smith AL, Andersen CL, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? Endocr Relat Cancer. 2012 Dec;19(6):R255–79.

25. Rodrìguez LV, Wang B, Shortliffe LMD. Structural changes in the bladder walls of pregnant and hormone-treated rats: correlation with bladder dynamics. BJU Int. 2004 Dec;94(9):1366–72.

26. Shen SS, Smith CL, Hsieh J-T, Yu J, Kim IY, Jian W, et al. Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue. Cancer. 2006 Jun;106(12):2610–6.

27. Blakeman PJ, Hilton P, Bulmer JN. Oestrogen and progesterone receptor expression in the female lower urinary tract, with reference to oestrogen status. BJU Int. 2000 Jul;86(1):32–8.

28. Thomas C, Gustafsson J-Å. The different roles of ER subtypes in cancer biology and therapy. Nat Rev Cancer. 2011 Aug;11(8):597.

29. Batra SC, Iosif CS. Progesterone receptors in the female lower urinary tract. J Urol. 1987 Nov;138(5):1301–4.

30. Bai Y, Wang X, Yang Y, Tang Y, Wang J, Han P. Parity and bladder cancer risk: a dose-response meta-analysis. BMC Cancer [Internet]. 2017 Jan [cited 2017 May 31];17. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5219774/

31. La Vecchia C, Negri E, Franceschi S, Parazzini F. Long-term impact of reproductive factors on cancer risk. Int J Cancer. 1993 Jan 21;53(2):215–9.

558 Table 1: Menstrual factors, and exogenous hormone use in relation to UC risk in EPIC Women.
559

		Overall	
	Person-years	**Cases (%) n=529**	**HR (95%CI) [a]**
Age at menarche, years			
<12	678 236	64 (12.1)	1.00 (referent)
12	955 271	103 (19.5)	1.10 (0.80- 1.51)
13	1 166 665	128 (24.2)	1.05 (0.78- 1.43)
14	976 383	108 (20.4)	0.92 (0.67- 1.26)
>14	718 342	113 (21.4)	1.07 (0.78- 1.48)
Unknown	166 304	13 (2.5)	
***P* trend**			0.845
Cumulative duration of menstrual cycling, accounting for OC use, years [b]			
<23	960 018	72 (13.6)	1.00 (referent)
23- <30	693 105	96 (18.2)	1.01 (0.73- 1.39)
30- <35	920 740	108 (20.4)	0.87 (0.63- 1.21)
≥35	805 979	142 (26.8)	1.00 (0.71- 1.40)
Unknown	1 011 360	111 (21.0)	1.05 (0.74- 1.48)
***P* trend**			0.924
Use of OC			
No	1 859 302	278 (52.6)	1.00 (referent)
Yes	2 668 828	239 (45.2)	0.93 (0.77- 1.14)
Unknown	133 072	12 (2.3)	
Duration OC use, years			
No	1 859 302	278 (52.6)	1.00 (referent)
>0- ≤1	495 753	34 (6.4)	0.70 (0.49- 1.01)
>1- 5	780 263	63 (11.9)	0.94 (0.71- 1.26)
>5- 10	594 859	69 (13.0)	1.22 (0.92- 1.63)
>10	546 567	51 (9.6)	0.82 (0.59- 1.13)
Unknown duration	251 386	22 (4.2)	
Missing use of OC	133 072	12 (2.3)	
***P* trend**			0.259
Menopausal status			
Premenopausal	1 654 703	49 (9.3)	1.00 (referent)
Perimenopausal	896 065	64 (12.1)	1.32 (0.77- 2.8)
Natural postmenopausal	1 992 700	394 (74.5)	1.88 (1.09- 3.25)
Surgical postmenopuasal	117 733	22 (4.2)	2.15 (1.10- 4.20)
Age at natural menopause, years [c]			
≤46	385 834	85 (21.6)	1.17 (0.87- 1.58)
47- 49	337 177	68 (17.3)	1.08 (0.79- 1.48)
50 - 52	509 460	97 (24.6)	1.00 (referent)
≥53	305 850	79 (20.1)	1.33 (0.99- 1.80)
Unknown	454 379	65 (16.5)	1.21 (0.86- 1.70)
***P* trend**			0.527
Age at any menopause, years			
≤46	450 220	100 (24.0)	1.21 (0.91- 1.60)
47- 49	360 268	70 (16.8)	1.04 (0.76- 1.42)
50 - 52	527 478	101 (24.3)	1.00 (referent)

≥53	315 160	80 (19.6)	1.31 (0.97- 1.77)
Unknown	457 307	65 (15.6)	1.20 (0.86- 1.68)
***P* trend**			0.853
Use of MHT [d]			
No	1 740 862	247 (51.5)	1.00 (referent)
Yes	1 072 357	172 (35.8)	1.28 (1.04- 1.58)
Unknown	193 278	61 (12.7)	1.32 (0.90- 1.95)
Duration MHT use, years [d]			
No	1 740 862	247 (51.5)	1.00 (referent)
>0- ≤1.25	321 348	51 (10.6)	1.33 (0.98- 1.81)
>1.25-4	336 578	47 (9.8)	1.37 (0.99- 1.90)
>4	310 366	56 (11.7)	1.27 (0.93- 1.73)
Unknown duration	104 065	18 (3.8)	
Unknown use of MHT	193 278	61 (12.7)	1.03 (0.74- 1.43)
***P* trend**			0.152
Type of MHT [d, e]			
Non-users of MHT	1 527 202	215 (58.0)	1.00 (referent)
Oestrogen alone	178 339	32 (8.6)	1.43 (0.97- 2.10)
Oestrogen + Progestin	527 153	50 (13.5)	1.08 (0.77- 1.51)
Unknown type of MHT	329 620	74 (20.0)	1.37 (1.04- 1.81)
Oophorectomy [f]			
No	3 407 081	344 (76.1)	1.00 (referent)
Unilateral	145 533	28 (6.2)	1.32 (0.90- 1.95)
Bilateral	131 175	23 (5.1)	1.12 (0.73- 1.72)
Unknown if unilateral or bilateral	11 831	2 (0.4)	
Unknown	965 580	55 (12.2)	0.91 (0.47- 1.78)
Hysterectomy [f]			
No	3 640 275	344 (76.1)	1.00 (referent)
Yes	472 260	76 (16.8)	1.09 (0.84- 1.40)
Unknown	548 667	32 (7.1)	

UC: Urothelial Carcinoma // OC: oral contraceptive // MHT: menopause hormone therapy

Estimations of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

[a] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake, OC use, and full-term pregnancies

[c] Women who had surgical menopause were excluded.

[d] In peri- and postmenopausal (natural or surgical).

[e] Available in France, Italy, Spain, United kingdom, The Netherlands, Germany, Denmark, and Norway.

[f] Available in all centres except Malmö.

572 Table 2: Reproductive factors in relation to UC risk in EPIC Women.
573

	Person-years	Cases (%) n=529	HR (95%CI)[a]
Parity			
No	686 624	73 (13.8)	1.00 (referent)
Yes	3 774 138	440 (83.2)	0.87 (0.68- 1.12)
Unknown	200 439	16 (3.0)	
Number of full-term pregnancies [b]			
0 [c]	686 624	69 (13.5)	0.92 (0.67- 1.25)
1	663 853	99 (19.4)	1.00 (referent)
2	1 787 539	192 (37.6)	0.80 (0.62- 1.02)
3	845 995	89 (17.4)	0.70 (0.52- 0.94)
4	253 868	35 (6.9)	0.79 (0.53- 1.18)
≥5	110 467	11 (2.2)	0.47 (0.25- 0.88)
Unknown parity	200 439	16 (3.1)	
***P*-trend[d]**			0.008
Age at first full-term pregnancy, years [d]			
≤20	546 150	68 (15.5)	1.00 (referent)
21- 23	1 001 554	119 (27.1)	1.03 (0.76- 1.40)
24- 25	742 124	73 (16.6)	0.86 (0.61- 1.20)
26- 30	1 086 162	139 (31.6)	1.03 (0.76- 1.39)
≥30	382 435	40 (9.1)	0.89 (0.59- 1.32)
Unknown	15 713	1 (0.2)	
P-trend			0.688
Breastfeeding [d, e]			
No	523 624	57 (14.1)	1.00 (referent)
Yes	2 984 829	341 (83.8)	0.85 (0.64- 1.14)
Unknown	63 513	9 (2.2)	
Duration of breastfeeding, all pregnancies, months [e, f]			
>0-≤3	854 602	115 (33.7)	1.00 (referent)
>3- 12	1 327 975	142 (41.6)	0.73 (0.56- 0.95)
>12	771 517	79 (23.2)	0.78 (0.55- 1.09)
Unknown	31 193	5 (1.5)	
P-trend			0.092
Induced abortions [g]			
Never pregnant	483 030	48 (12.4)	1.19 (0.91- 1.56)
0	2 466 069	269 (69.7)	1.00 (referent)
1	404 767	45 (11.7)	1.12 (0.81- 1.56)
≥2	176 646	19 (4.9)	1.01 (0.62- 1.64)
Unknown	69 032	5 (1.3)	
***P*-trend**			0.759
Spontaneous abortions [h]			
Never pregnant	508 626	56 (12.1)	1.14 (0.85- 1.52)
0	2 469 123	295 (63.7)	1.00 (referent)
1	587 558	78 (16.9)	1.10 (0.86- 1.42)
≥2	200 186	27 (5.8)	1.05 (0.71- 1.56)
Unknown	73 119	7 (1.5)	
***P*-trend**			0.497
Infertility problems [i]			

	No	2 872 888	255 (83.3)	1.00 (referent)
	Yes	142 531	16 (5.2)	1.61 (0.97- 2.69)
	Unknown	151 702	35 (11.4)	1.72 (0.24- 12.51)

UC: Urothelial Carcinoma

Estimations of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

[a] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b] Available in all centres except Bilthoven.

[c] Including nulliparous women and women without full-term pregnancies.

[d] In parous women.

[e] Available in all centres except Bilthoven and Umeå.

[f] In parous women who has ever breastfed.

[g] Available in all centres except Bilthoven, Malmö, Umeå, and Norway.

[h] Available in all centres except Bilthoven, Umeå, and Norway.

[i] Available in France, Italy, Spain, United Kingdom, Utrecht, Greece, and Germany.

Table 3: Mutually adjusted models for menopause status, MHT, and parity, and UC

	Overall		Never smokers	
	Cases (%)	**HR (95%CI)[a]**	**Cases (%)**	**HR (95%CI)[b]**
Full cohort	n=529		n=195	
Menopausal status & use of MHT				
Premenopausal	49 (9.26)	0.73 (0.43- 1.22)	18 (9.23)	1.23 (0.52- 2.43)
Peri-/Postmenopausal & non-users of MHT	247 (46.7)	1.00 (referent)	105 (53.9)	1.00 (referent)
Peri-/Postmenopausal & users of MHT	172 (32.5)	1.27 (1.03- 1.57)	52 (26.7)	1.02 (0.71- 1.47)
Peri-/Postmenopausal & unknown MHT-use	61 (11.5)	1.35 (0.88- 2.07)	20 (10.26)	1.12 (0.53- 2.39)
Number of full-term pregnancies [c]				
0 [d]	69 (13.5)	0.92 (0.67- 1.25)	19 (9.7)	0.72 (0.40- 1.29)
1	99 (19.4)	1.00 (referent)	32 (16.4)	1.00 (referent)
2	192 (37.6)	0.80 (0.62- 1.02)	83 (42.6)	0.95 (0.63- 1.45)
3	89 (17.4)	0.70 (0.52- 0.94)	39 (20.0)	0.85 (0.52- 1.37)
4	35 (6.9)	0.80 (0.54- 1.19)	9 (4.6)	0.57 (0.27- 1.21)
≥5	11 (2.2)	0.48 (0.25- 0.90)	5 (2.6)	0.49 (0.18- 1.29)
Unknown parity	16 (3.1)		8 (4.1)	
Information not available	18			
***P*-trend [e]**		0.010		0.069

UC: Urothelial Carcinoma // MHT: menopausal hormone therapy

[a] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by menopausal status and MHT, number of full-term pregnancies, smoking status and intensity, fruits and vegetables intake.

[b] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by menopausal status and MHT, number of full-term pregnancies, fruits and vegetables intake.

[c] Available in all centres have information except Bilthoven.

[d] Including nulliparous women and women without full-term pregnancies.

[e] In parous women

7. DISCUSSION

Each paper of this doctoral thesis includes a discussion section. In the next section, a global discussion of the main results is presented. The discussion is organized in two sections: 1) lifestyle, diet, and reproductive history at earlier age at natural menopause risk, and 2) menstrual factors, reproductive history, and exogenous hormones in pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma risks.

7.1. Lifestyle, diet, and reproductive history in earlier age at natural menopause risk

We have evaluated the role of lifestyle, diet, and reproductive history in the risk of earlier age at natural menopause in the largest prospective cohort in Spain. In our study, we observed a median age at natural menopause of 51 years. Our results confirm the association between tobacco smoking and earlier age at natural menopause. Later ages at natural menopause were observed in those women who had irregular menses the first 10 years after menarche, who use OC, and have higher number of total pregnancies (including full-term pregnancies and abortions). The observed relations remained significant after mutually adjusted for smoking status, regular menses, history of OC use and age at start of OCs, and the number of pregnancies. Each block of factors is separately discussed in detail below.

7.1.1. Lifestyle factors and age at natural menopause

Our results in tobacco use are in agreement with the literature (7). We observed a 29% increased risk of having an earlier age at natural menopause in women who reported that they smoked tobacco at baseline. Cigarette smoking has an anti-estrogenic effect on women and is considered an important sex hormone modifier (102). It has been suggested that the effect of smoking on age at natural menopause is reversible if woman quit smoking during her earlier reproductive life (103), but unexpectedly we did not find a significant reduction in former smokers. Passive smoking exposure has been less studied and may be also associated with earlier age at natural menopause (104). Unfortunately, passive smoking information is not available in the EPIC-Spain cohort.

Inconsistent results in relation to BMI and age at natural menopause were observed in the literature (34); although a weak association was observed in a meta-analysis between women with overweight and later age at natural menopause compared to women with normo-weight. This relationship may be to higher estrogen levels in adipose tissue (105). Inconsistencies in the results may be explained by differences in the study design and in the adjustment for potential confounder variables, especially tobacco smoking which is the main predictor of earlier age at natural menopause. Our results were not able to clarify if BMI plays a role in age at natural menopause. We observed no statistically significant inverse association between overweight and obese women and age at natural menopause. In the Spanish-EPIC cohort most of the women were classified as obese or overweight (n=8,018, 63.8%), 36% as normal weight and 0.2% as underweight. Since the publication of our results, the InterLACE consortium (including 11 prospective studies and 24,196 postmenopausal women) has shown that being underweight may increase the risk of an earlier age at natural menopause, while being overweight or obese may delay the onset of menopause (106). Based on the published literature and our results, the role of BMI in age at natural menopause is still unclear. Further cohort studies, including a large variability in women's BMI, will be need to clarify, if any, the effect of BMI on age at natural menopause.

The EPIC-PANACEA study (European Prospective Investigation into Cancer-Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating out of home And Obesity) has found that physical activity and educational level were inversely associated with BMI (107), thus our results for active life, and university studies and age at natural menopause were in concordance with our results for overweight and obese women and age at natural menopause. A few studies have shown that higher overall physical activity was weakly associated with later age at natural menopause (42,108,109), others (including our) observed no association (41,110,111). Emaus *et al.* (110) and Zhao *et al.* (111) found that the effect of high physical differed by smoking status. Emaus *et al.* (110) observed later age at natural menopause in former heavy smokers (HR: 0.88; 95% CI: 0.81-0.97); on the contrary, Zhao *et al.* (111) detected a later age at natural menopause in never smokers (HR: 0.77; 95% CI: 0.63- 0.95). Overall, this suggests that physical activity does not have an important role in regard to the risk of earlier age at natural menopause.

7.1.2. Dietary factors and age at natural menopause

A few studies in European women have examined dietary habits (41–43). A previous study showed an association between earlier age at natural menopause and higher intake of vegetables, carbohydrates, fiber, soy products, and cereal products; while later age at natural menopause was associated with higher consumption of fat, meat, and protein (41). No effect on menopause of fats and carbohydrate was reported in a randomized clinical trial (low-fat, high-carbohydrate [LFHC] dietary intervention) in women with greater risk of breast cancer (43). However, higher intakes of vegetables were associated with later age at natural menopause in Central and Eastern European urban populations (42). A recent study found that later age at natural menopause was associated with high intakes of oily fish and fresh legumes; while earlier age at natural menopause was related to a high consumption of refined pasta/rice (112). Finally, vegetarian diet may be associated with an earlier age at natural menopause (112,113).

Our results on diet showed no association between dietary habits assessed after the age of 40 years and age at natural menopause. Furthermore, no association was observed between arMED index (characterized by elevated intakes of fruit and vegetables) and age at natural menopause. Longer exposures to foods and nutrients with known estrogenic potential (such as soy, fiber, fats, vitamin D, polyphenols, and nuts) should be examined.

7.1.3. Reproductive factors and age at natural menopause

Our null results on age at menarche, age at first full-term pregnancy, and history of breastfeeding in relation to age at natural menopause are in concordance with the literature (34). In agreement with other studies (41,114), we observed that women who had irregular menses during the first 10 years after menarche might delay age at natural menopause. As mentioned previously, the first few years after menarche, menstrual periods are usually irregular and anovulatory. Long irregular menstrual periods are also due to polycystic ovary syndrome (PCOS), and almost 10% of women are affected by PCOS worldwide. It has been seen that women with PCOS have longer reproductive life spans (115,116), which could explain our results on time to regular menses. OC use is usually prescribed to regulate menstrual cycles. Overall, we observed that women who had use OC were older at the moment of menopause, especially women who

started OC use between the ages of 25 and 30 years compared to those who started after 31 years. Based on the "Doorlopend Onderzoek Morbiditeit/Mortaliteit" (DOM-3) cohort results (117), we performed the analysis by OC dose; and on the contrary to their results we observed that short-term users of high doses OCs had a reduced risk of earlier age at natural menopause compared to non-users. These findings support the hypothesis that a reduction in FHS and LH may delay age at natural menopause. In the EPIC-cohort, long-term users of high OC doses may have changed their doses to a lower one and therefore, the potential effect on age at natural menopause might have been attenuated. Based on the reviewed literature and our results, we can presume that women with irregular menses the first years after menarche undergo later menopause. The prescription of OC to regulate menstrual periods could explain this association.

Finally, we observed that women with more than one pregnancy (including full-term pregnancies and abortions) were older at the moment of menopause in agreement with the literature (42,43,108,113,118,119). The absence of ovulation during pregnancy could delay oocytes depletion, which may delay natural menopause (34).

Little information has been published in relation to premature ovarian failure and its relation to unilateral oophorectomy. It has been found that age at natural menopause occurs approximately between 1 to 2 years earlier in women with unilateral oophorectomy compared to those with intact ovaries (51,120). We observed no association between unilateral oophorectomy and age at natural menopause.

7.2. Menstrual factors, reproductive history, and exogenous hormones in pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma risks

We have assessed the relation between menstrual factors, reproductive history, and exogenous hormones and pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma risks in a large international case-control consortium (PANC4) and a large European cohort (EPIC). Our results provided little support to the hypothesis that sex hormones play a role in either pancreatic cancer or non-Hodgkin lymphoma. However, strong evidence of the role of parity in urothelial carcinogenesis was observed. We observed no statistically significant associations between these factors and the risk of either non-Hodgkin lymphoma subtypes or urothelial carcinoma tumor grades or

urothelial carcinoma tumor aggressiveness. Each block of factors will be discussed in detail below.

7.2.1. Menstrual factors

Our results are in agreement with the literature and do not support the hypothesis that either earlier menarche nor later age at natural menopause influence the risk of the studied cancers. Thus, longer exposure to endogenous hormones through an earlier age at menarche and/or a later age at menopause does not change the risk of these cancers.

7.2.2. Reproductive history

Women experience several hormonal changes during pregnancy, including an increase in both estrogen and progesterone levels. Furthermore, hormonal changes continue during breastfeeding. We hypothesized that pregnancy increases hormone levels and therefore protects against pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma. Scattered reports of associations between parity and pancreatic cancer have been published. Consistent results were observed in relation to parity and urothelial carcinoma and non-Hodgkin lymphoma risks. No associations were found between age at first full-term pregnancy and breastfeeding, and the studied cancers.

Two meta-analyses (both published in 2014) attempted to elucidate the relations between parity and pancreatic cancer risk. Some differences in the included studies were found between both meta-analyses. One found an inverse linear trend between number of full-term pregnancies and pancreatic cancer risk (76). The other one observed that giving birth to twice reduce the risk of pancreatic cancer, but they did not observe a linear trend between number of full-term pregnancies and the risk of pancreatic cancer (77). Our results are in agreement with most of recent publications (121–123), showing no association between number of the full-term pregnancies (including live births and stillbirths) or number of pregnancies (including number of full-term pregnancies and abortions), and the risk of pancreatic cancer.

We observed null findings in relation to reproductive history and non-Hodgkin lymphoma. Findings from the pooled analysis of the International Lymphoma Epidemiology Consortium (InterLymph; including 18 case-control studies with 3,816 cases and 5,151 controls) (124) and from a systematic review (74) were also nulls,

concluding that the increased levels of estrogens during pregnancy does not explain the reduction of the non-Hodgkin lymphoma risk in women.

Previous prospective studies (79–81) and two meta-analyses (82,83) observed a reduced risk of urothelial carcinoma in parous women, independent of the number of births (79,81–83,86,88,125). Contrary, Weibull *et al.* (80) and our findings showed an association between higher number of full-term pregnancies and a lower risk of urothelial carcinoma. The main difference between these results is the category of reference; while most of the studies that found no linear trend used "nulliparous" as a referent category, Weibull *et al.* and us used "one birth". Nulliparous women are more likely to represent a heterogeneous group that includes women with and without fertility problems. It has been suggested that progesterone suppresses ER expression (expressed in both normal and cancerous urothelial cells) during pregnancy (126). Thus, it can be hypothesized that these increased levels of both estrogen and progesterone may reduce urothelial carcinoma risk in parous women.

In summary, reproductive history does not influence on the risk of developing pancreatic cancer and non-Hodgkin lymphoma. Women with more than one birth may be at lower risk of urothelial carcinoma

7.2.3. Oophorectomy and hysterectomy

We observed no association between oophorectomy and hysterectomy and urothelial carcinoma risk. Nevertheless, our results suggested a lower risk of pancreatic cancer in hysterectomized women and a higher risk of non-Hodgkin lymphoma in women with either hysterectomy or bilateral oophorectomy.

Epidemiologic evidence on the role of oophorectomy and hysterectomy in the studied cancers is still scarce. In relation to pancreatic cancer, the Iowa Women's Health Study (IWHS) cohort observed an increase risk with both hysterectomy and bilateral oophorectomy (86). The Cancer Prevention Study-II Nutrition Cohort found an increased pancreatic cancer risk associated with hysterectomy (with intact ovaries) compared to no surgery; while no effect was observed with hysterectomy with bilateral oophorectomy (52). In the EPIC cohort and the WHI, no association was observed between either oophorectomy or hysterectomy and pancreatic cancer risk (121,127).

Some diseases, for which usual treatment is hysterectomy, are directly associated with increased female hormone levels (such as fibroids and hyperplasia). Unfortunately, the specific reason for having a hysterectomy was not available in PanC4 data, so we could not verify if the observed protective effect that was shown with hysterectomy was due to underlying elevated estrogen levels in the mentioned diseases or to other factors related to having a hysterectomy.

Regarding non-Hodgkin lymphoma, the California Teachers Study (CTS) cohort observed that women with bilateral oophorectomy and who had never used any MHT had a significant higher non-Hodgkin lymphoma risk (128). Furthermore, in a case-control study that evaluated multiple myeloma subtypes, surgical menopause (i.e. hysterectomy and bilateral oophorectomy) was associated with higher risk of multiple myeloma (129); but this association was not observed in the CTS cohort (129). A population-based case-control study found null results for non-Hodgkin lymphoma risk in relation to hysterectomy and oophorectomy (130).

The positive association that we observed between hysterectomy and bilateral oophorectomy and non-Hodgkin lymphoma risk was based on a small number of cases, and the estimate for oophorectomy without a hysterectomy was below 1, which hampered the biological interpretation of the exposure. Furthermore, our result could simply reflect type 1 error, given the large number of factors evaluated in the analyses. Pooled analyses of cohort studies may provide the statistical power needed to rule out the role, if any, of hysterectomy and oophorectomy in the risk of non-Hodgkin lymphoma.

Finally, an increased risk of urothelial carcinoma in women with bilateral oophorectomy compared to women with intact ovaries was observed in the IWHS (86). A population-based case-control study observed that women who underwent a bilateral oophorectomy at earlier ages (<45 years) were at higher risk of developing urothelial carcinoma than those who underwent bilateral oophorectomy after 45 years (83). However, Davis-Dao *et al.* (82) observed no association between urothelial carcinoma and bilateral oophorectomy. In general, no association has been observed between hysterectomy and urothelial carcinoma risk (79,82,121).

Based on our results and previous results, we cannot confirm that either hysterectomy or oophorectomy play a role in the carcinogenesis of pancreatic cancer, non-Hodgkin lymphoma, or urothelial carcinoma.

7.2.4. Exogenous hormones

7.2.4.1. Hormonal contraception

Most of the previous studies, including ours, observed no association between OC use and the risks of both pancreatic cancer and urothelial carcinoma. However, the CTS study found that a longer exposure (≥10 years) to OC increased to 72% the risk of developing pancreatic cancer compared to never OC users (131). Recently, the WHI study observed no association between OC use and duration of OC use and pancreatic cancer risk (121). Furthermore, a large cohort study among premenopausal women (132) concluded that the use of total OC and any the type of OC did not influence on the risk of pancreatic cancer. This study included all women living in Denmark aged between 15 and 49 years at the begging of 1995 and collected information from the Danish Cancer Register, the National Register of Medical Product Statistics (which provided information on OC use), and the National Birth Register.

Results on OC use and non-Hodgkin lymphoma risk are inconsistent depending on the study and non-Hodgkin lymphoma subtype. We observed null results between OC use and non-Hodgkin lymphoma risk, as in two cohort studies (133,134); although a longer duration of OC use was related to lower risk in the diffuse large B-cell lymphoma subtype (133). The CTS study found that women who start OC use at 25 years old were at lower risk of non-Hodgkin lymphoma compared to never users (128). The InterLymph (including 9 case-control studies) found no association between overall non-Hodgkin lymphoma and OC use, but an increased risk in the follicular lymphoma subtype (124). Finally, the EpiLymph study (European case-control study) observed that a shorter duration of OC use, starting OC use after 25 years old, the longer mean time after the last OC use, or starting OC use between 1970-79 were associated with a higher risk of non-Hodgkin lymphoma (especially diffuse large B-cell lymphoma subtype, follicular lymphoma subtype, and Chronis lymphocytic leukemia / small lymphocytic leukemia compared to never users (135).

7.2.4.2. Menopausal hormone therapy

We observed no association between MHT and pancreatic cancer risk; however, women who had a hysterectomy and had used MHT were at reduced pancreatic cancer risk. Generally, no association between MHT and pancreatic cancer risk was found in the literature (127,136–139). No other studies have analyzed the joint effect of hysterectomy and MHT. In our analysis, we were not able to distinguish the type of MHT; however, estrogen-only MHT is usually recommended for women who have had a hysterectomy. Two studies found that users of estrogen-only MHT had a reduced risk of pancreatic cancer compared to never users (123,131), although both studies were based on a small number of cancer cases. However, the WHI, based on a larger number of pancreatic cancer cases, observed no association between MHT and any type of MHT, and the risk of pancreatic cancer (121).

Our results on the relation between total and by subtype of MHT and non-Hodgkin lymphoma risk were null; although contradictory findings were observed in the literature. In case-control studies, an inverse association between MHT and non-Hodgkin lymphoma risk was detected (124,130,140–143); while in cohort studies association were observed (128,133,134,144,145). A systematic review of previous literature suggested that the association between MHT and non-Hodgkin lymphoma may depend on the MHT formulation and oophorectomy status (74). Nevertheless, in the WHI, the risk of non-Hodgkin lymphoma was similar in the MHT-users and the non-users (146).

We observed a higher urothelial carcinoma risk among MHT users compared to non-users, contrary to previous studies which found no relation between them (81–83). Since we did not observe the association in never-smokers, and the overall MHT effect only remained significant in ever-smokers, residual confounding from tobacco smoking is a possible explanation for our MHT results. A meta-analysis (based on 4 cohort studies) of MHT, by formulation (estrogen or estrogen plus progestin), showed a 39% decreased urothelial carcinoma risk in users of estrogen plus progestin, while no effect was found with the use of estrogen alone (79). The WHI found no influence on of the formulation of MHT on the urothelial carcinoma risk (81).

Overall, our and other cohort data suggest that exogenous hormones are not associated with pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma.

7.3. Strengths and limitations

Our study on age at natural menopause contain the largest number of subjects evaluated to date to study lifestyle, diet, menstrual factors and reproductive history, and age at natural menopause in five Spanish regions. To evaluate associations on hormonal factors and the studied cancers, we used two large datasets (PanC4 consortium and EPIC study) which allowed us to evaluate the role of hormones in non-Hodgkin lymphoma, by histological subtypes, and in urothelial carcinoma, by urothelial carcinoma subtypes, being the first study taking into account urothelial carcinoma subtypes. We could control for potential confounders at baseline (such as education and BMI) which might have biased previous studies of MHT and diseases due to a healthy user effect (147). However, these variables may imprecisely measure complex factors such as socioeconomic or adiposity, and therefore residual confounding cannot completely be ruled out.

In our studies we used self-reported questionnaires to collect reproductive information which can affect the reliability of exposure because of recall bias. In general, women tend to report correctly the number of births (148). Menopause is a prolonged biological event which can last one year or more, so accurate ages at menopause are more common in women with surgical than with natural menopause. However, some studies have shown that age at natural menopause was self-reported correctly within 1 year (148–150). Self-reported information on OC use has been shown to be comparable with pharmacy reports; although, it is preferable to use pharmacy reports for former OC-users and interviews for current OC-users (151). Finally, >90% of self-reported hysterectomy, oophorectomy, and HRT cases were in accordance between baseline and follow-up interview in a cohort of Portuguese adults (148).

We would also like to highlight that information on reproductive history and exogenous hormone use was available only at cohort enrolment; however, we noted that 78.7% of the cases were postmenopausal at recruitment, so reproductive history was essentially complete for most participants. Moreover, we observed similar results for the final mutually-adjusted models in analyses restricted to postmenopausal women as we

observed for all study participants. Another potential limitation of our studies is the large number of missing values in the MHT variables (duration and formulation). Moreover, information on MHT was not periodically updated, and therefore, we could not evaluate risk in women who started using MHT or modified their use after enrolment.

In the analysis of age at natural menopause, we excluded participants with ages <35 years at recruitment, that we considered at lower risk of undergoing menopausal during the three years of follow-up. Hereby, we excluded women with premature ovarian failure; although based on the Spanish fertility association, premature ovarian failure prevalence in European women is only 1%. We repeated the final table of the paper including women with ages <35 years and the results were essentially the same (Table 7). In this study, we have relatively short follow-up at the end of the fertile life span to evaluate the influence of past diet on age at natural menopause.

Table 7: Sensitivity analysis of the mutually adjusted model including women with ages <35 years at recruitment

	Post-menopausal	HR (95%CI)[a]	*P* for trend
Smoking status			
Never	934	Reference	
Former	67	0.78 (0.61- 1.01)	
Current	151	1.29 (1.08- 1.55)	
Time since regular menses			
Always regular	1002	Reference	0.004
After 1 years	45	0.95 (0.70- 1.30)	
After 2-5+ years	27	0.86 (0.58- 1.26)	
Always irregular the 1st 10 years	74	0.70 (0.55- 0.90)	
OC use and start (years)			
Never use	760	1.32 (1.01- 1.57)	0.009
<25	52	1.16 (0.84- 1.60)	
25-30	163	Reference	
≥ 31	173	1.42 (1.14- 1.76)	
Number of pregnancies			
Never pregnant	104	Reference	0.027
1	74	0.91 (0.67- 1.24)	
2	297	0.76 (0.61- 0.97)	
3	333	0.83 (0.65- 1.04)	
≥4	340	0.74 (0.56- 0.94)	

[a] HR > 1 indicates that the risk factor was associated with an earlier ANM and a HR <1 indicates that the risk factor was associated with a later ANM. Mutually adjusted Cox model stratified by center and age at recruitment

7.4. Benefits of research, applicability, and future research lines

This doctoral thesis provides comprehensive results on the role of lifestyle and reproductive factors in the age at natural menopause. Further, it provides unique epidemiological evidence on the relationships between reproductive factors and exogenous hormones use, and the risk of pancreatic cancer, non-Hodgkin lymphoma, and urothelial carcinoma of the bladder.

These results can help to detect women with increased risk of chronic diseases (such as osteoporosis and cardiovascular disease) associated with earlier age at menopause and strengthens the possibility of early preventive strategies and clinical surveillance. Further investigations on the role of modifiable factors (including also dietary habits in earlier life, second-hand tobacco smoke exposure, and exposure to environmental contaminants) in self-reported age at natural menopause and the use of serum FSH and LH levels to more accurately diagnose menopause status are needed.

Based on our findings, reproductive factors, and exogenous hormones do not seem to substantially contribute to the carcinogenesis of pancreatic cancer and non-Hodgkin lymphoma. However, our results confirm the increasing benefit of having more than one birth on urothelial carcinoma risk. Our results could provide additional information for early detection of urothelial cancer, taking into account that nulliparous women on their sixties are a higher risk of developing urothelial cancer. Results from other large cohorts and consortia with a large sample of never-smokers, might help to clarify the evidence provided by this analysis. More studies on number of full-term pregnancy are needed to elucidate the putative “protective” effects of parity. Further investigations of the role of perinatal hormonal changes and how these changes may affect to ER and PR levels and urothelial cells in the bladder are also needed.

8. CONCLUSIONS

The conclusions are present as response to each hypothesis enumerated at the beginning of this doctoral thesis:

Hypothesis 1: *Lifestyle factors (including educational level, BMI, physical activity, and tobacco), diet, and menstrual and reproductive history, and oral contraceptive use influence on age at natural menopause.*

- Current smokers increased 29% the risk of an earlier age at natural menopause.
- We observed no statistically significant inverse association between being overweight and later age at natural menopause.
- Physical activity and educational level do not have an important role in regard to the risk of an earlier menopause.
- We observed no effect of dietary habits assessed after the age of 40 years on age at natural menopause.
- We observed that women with a longer mean time from menarche and regular menses, those who have history of OC, and women with more than one pregnancy were older at the onset of menopause.

Hypothesis 2: *Menstrual factors, reproductive history, and hormone use are protective factors of pancreatic cancer, Non-Hodgkin lymphoma, and Urothelial carcinoma.*

1. *Earlier age at menarche, later age at menopause, parity, and heaving history of hormone use are protective against pancreatic cancer.*

- Menstrual factors, reproductive history, and hormone are not associated with the risk of pancreatic cancer.

2. *Parity, later age at menopause and heaving history of hormone use are protective against Urothelial carcinoma.*

- We observed an increasing benefit of each birth after the first on urothelial carcinoma risk.
- Our study suggests that MHT do not play any role in the risk of urothelial carcinoma.

3. *Parity and heaving history of hormone use are protective against Non-Hodgkin lymphoma.*

- Menstrual factors, reproductive history, and hormone use are not associated with the risk of non-Hodgkin lymphoma.

9. REFERENCES

1. Cancer today [Internet]. [cited 2019 Apr 4]. Available from: http://gco.iarc.fr/today/home

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015 Feb;65(1):5–29.

3. Gong Z, Holly EA, Bracci PM. Survival in population-based pancreatic cancer patients: San Francisco Bay area, 1995-1999. Am J Epidemiol. 2011 Dec;174(12):1373–81.

4. European Cancer Information System [Internet]. [cited 2019 Apr 24]. Available from: https://ecis.jrc.ec.europa.eu/explorer.php?$0-2

5. Yeo TP, Lowenfels AB. Demographics and epidemiology of pancreatic cancer. Cancer J Sudbury Mass. 2012 Dec;18(6):477–84.

6. Kristin E. Anderson, Thomas M. Mack, Debra T. Silverman. Cancer of the Pancreas. In: David Schottenfeld, Joseph F. Fraumeni, editors. Cancer Epidemiology and Prevention. Third. New York: Oxford University Press; 2006. p. 721–62.

7. Wolpin BM, Rizzato C, Kraft P, Kooperberg C, Petersen GM, Wang Z, et al. Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. Nat Genet. 2014 Sep;46(9):994–1000.

8. Iodice S, Gandini S, Maisonneuve P, Lowenfels AB. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. Langenbecks Arch Surg Dtsch Ges Für Chir. 2008 Jul;393(4):535–45.

9. Perry AM, Jacques D, Nathwani BN, Maclennan KA, Müller-Hermelink HK, Boilesen E, et al. Classification of Non-Hodgkin Lymphoma in Seven Geographic Regions Around the World: Review of 4539 Cases from the International Non-Hodgkin Lymphoma Classification Project. Blood. 2015 Dec;126(23):1484–1484.

10. Non-Hodgkin Lymphoma - Cancer Stat Facts [Internet]. [cited 2019 Apr 24]. Available from: https://seer.cancer.gov/statfacts/html/nhl.html

11. Armitage JO, Gascoyne RD, Lunning MA, Cavalli F. Non-Hodgkin lymphoma. Lancet Lond Engl. 2017 Jul;390(10091):298–310.

12. Morton LM, Slager SL, Cerhan JR, Wang SS, Vajdic CM, Skibola CF, et al. Etiologic Heterogeneity Among Non-Hodgkin Lymphoma Subtypes: The InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014 Aug;2014(48):130–44.

13. Malats N, Real FX. Epidemiology of bladder cancer. Hematol Oncol Clin North Am. 2015 Apr;29(2):177–89, vii.

14. Roswall N, Freisling H, Bueno-de-Mesquita HB, Ros M, Christensen J, Overvad K, et al. Anthropometric measures and bladder cancer risk: a prospective study in the EPIC cohort. Int J Cancer. 2014 Dec;135(12):2918–29.

15. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, et al. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016." Eur Urol Focus. 2018 Jan 20;5(3):457–66.

16. Bladder cancer statistics | World Cancer Research Fund International [Internet]. [cited 2017 Apr 11]. Available from: http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/bladder-cancer-statistics

17. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011 Aug;306(7):737–45.

18. Jameson JL. Harrison's Endocrinology, Second Edition. McGraw Hill Professional; 2010. 560 p.

19. Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [cited 2019 Apr 25]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK279054/

20. Messinis IE. From Menarche to Regular Menstruation. Ann N Y Acad Sci. 2006;1092(1):49–56.

21. Parent A-S, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon J-P. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. Endocr Rev. 2003 Oct;24(5):668–93.

22. Mishra GD, Chung H-F, Pandeya N, Kuh D, Hayashi K, Lee JS, et al. Variations in reproductive events across life: a pooled analysis of data from 505 147 women across 10 countries. Hum Reprod. 2019 May;34(5):881–93.

23. Charalampopoulos D, McLoughlin A, Elks CE, Ong KK. Age at menarche and risks of all-cause and cardiovascular death: a systematic review and meta-analysis. Am J Epidemiol. 2014 Jul;180(1):29–40.

24. Modugno F, Laskey R, Smith AL, Andersen CL, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? Endocr Relat Cancer. 2012 Dec;19(6):R255–79.

25. McNeilly AS. Effects of lactation on fertility. Br Med Bull. 1979 May;35(2):151–4.

26. Mesiano S. Chapter 11 - Endocrinology of Human Pregnancy and Fetal-Placental Neuroendocrine Development. In: Strauss JF, Barbieri RL, editors. Yen and Jaffe's Reproductive Endocrinology (Eighth Edition) [Internet]. Philadelphia: Content Repository Only!; 2019 [cited 2019 Apr 26]. p. 256-284.e9. Available from: http://www.sciencedirect.com/science/article/pii/B9780323479127000111

27. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ. 2000 Jun;320(7251):1708–12.

28. de la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod Oxf Engl. 2002 Jun;17(6):1649–56.

29. Magnus MC, Wilcox AJ, Morken N-H, Weinberg CR, Håberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. BMJ. 2019 Mar;364:l869.

30. Thun M, Linet MS, Cerhan JR, Haiman CA, Schottenfeld D. Cancer Epidemiology and Prevention. Oxford University Press; 2017. 3681 p.

31. Slepicka PF, Cyrill SL, Dos Santos CO. Pregnancy and Breast Cancer: Pathways to Understand Risk and Prevention. Trends Mol Med. 2019 Oct;25(10):866–81.

32. WHO | Breastfeeding [Internet]. WHO. [cited 2019 Aug 2]. Available from: http://www.who.int/topics/breastfeeding/en/

33. Lactation (breastfeeding) [Internet]. World Cancer Research Fund. 2018 [cited 2019 Aug 2]. Available from: https://www.wcrf.org/dietandcancer/exposures/lactation-breastfeeding

34. Gold EB. The timing of the age at which natural menopause occurs. Obstet Gynecol Clin North Am. 2011 Sep;38(3):425–40.

35. Hvidman HW, Bentzen JG, Thuesen LL, Lauritsen MP, Forman JL, Loft A, et al. Infertile women below the age of 40 have similar anti-Müllerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. Hum Reprod Oxf Engl. 2016 May;31(5):1034–45.

36. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update. 2014 Oct;20(5):688–701.

37. Menopause Glossary, Menopause Resources | The North American Menopause Society, NAMS [Internet]. [cited 2017 Feb 15]. Available from: https://www.menopause.org/for-women/menopause-glossary

38. Daan NMP, Fauser BCJM. Menopause prediction and potential implications. Maturitas. 2015 Nov;82(3):257–65.

39. Sun L, Tan L, Yang F, Luo Y, Li X, Deng H-W, et al. Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. Menopause. 2012 Feb;19(2):126–32.

40. Taneri PE, Kiefte-de Jong JC, Bramer WM, Daan NMP, Franco OH, Muka T. Association of alcohol consumption with the onset of natural menopause: a systematic review and meta-analysis. Hum Reprod Update. 2016 Jun;22(4):516–28.

41. Nagel G, Altenburg H-P, Nieters A, Boffetta P, Linseisen J. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. Maturitas. 2005 Dec;52(3–4):337–47.

42. Stepaniak U, Szafraniex K, Kubinova R, Malyutina S, Peasey A, Pikhart H, et al. Age at natural menopause in three Central and Eastern European urban populations: The HAPIEE study. Maturitas. 2013;75(1):87–93.

43. Martin LJ, Greenberg CV, Kriukov V, Minkin S, Jenkins DJA, Boyd NF. Intervention with a low-fat, high-carbohydrate diet does not influence the timing of menopause. Am J Clin Nutr. 2006 Oct;84(4):920–8.

44. NIH Fact Sheets - Uterine Fibroids [Internet]. National Institutes of Health. 2013 [cited 2015 May 18]. Available from: http://report.nih.gov/nihfactsheets/viewfactsheet.aspx?csid=50

45. Hysterectomy [Internet]. Medscape. 2015 [cited 2015 May 18]. Available from: http://emedicine.medscape.com/article/267273-overview#a0103

46. Lacey JV, Chia VM, Rush BB, Carreon DJ, Richesson DA, Ioffe OB, et al. Incidence rates of endometrial hyperplasia, endometrial cancer and hysterectomy from 1980 to 2003 within a large prepaid health plan. Int J Cancer. 2012 Oct;131(8):1921–9.

47. Hammer A, Rositch AF, Kahlert J, Gravitt PE, Blaakaer J, Søgaard M. Global epidemiology of hysterectomy: possible impact on gynecological cancer rates. Am J Obstet Gynecol. 2015 Jul;213(1):23–9.

48. Trabuco EC, Moorman PG, Algeciras-Schimnich A, Weaver AL, Cliby WA. Association of Ovary-Sparing Hysterectomy With Ovarian Reserve. Obstet Gynecol. 2016 May;127(5):819–27.

49. Farquhar CM, Sadler L, Harvey SA, Stewart AW. The association of hysterectomy and menopause: a prospective cohort study. BJOG Int J Obstet Gynaecol. 2005;112(7):956–62.

50. Cooper GS, Thorp JM. FSH levels in relation to hysterectomy and to unilateral oophorectomy. Obstet Gynecol. 1999 Dec;94(6):969–72.

51. Rosendahl M, Simonsen MK, Kjer JJ. The influence of unilateral oophorectomy on the age of menopause. Climacteric J Int Menopause Soc. 2017 Dec;20(6):540–4.

52. Gaudet MM, Gapstur SM, Sun J, Teras LR, Campbell PT, Patel AV. Oophorectomy and hysterectomy and cancer incidence in the Cancer Prevention Study-II Nutrition Cohort. Obstet Gynecol. 2014 Jun;123(6):1247–55.

53. Zamora-Ros R, Rinaldi S, Biessy C, Tjønneland A, Halkjaer J, Fournier A, et al. Reproductive and menstrual factors and risk of differentiated thyroid carcinoma: the EPIC study. Int J Cancer. 2015 Mar;136(5):1218–27.

54. Luoto R, Auvinen A, Pukkala E, Hakama M. Hysterectomy and subsequent risk of cancer. Int J Epidemiol. 1997 Jun;26(3):476–83.

55. Guenego A, Mesrine S, Dartois L, Leenhardt L, Clavel-Chapelon F, Kvaskoff M, et al. Relation between hysterectomy, oophorectomy and the risk of incident differentiated thyroid cancer: The E3N cohort. Clin Endocrinol (Oxf). 2019;90(2):360–8.

56. United Nations Population Division | Department of Economic and Social Affairs [Internet]. [cited 2019 May 13]. Available from: https://www.un.org/en/development/desa/population/publications/family/contraceptive-wallchart-2013.asp

57. Barbaglia G, Macià F, Comas M, Sala M, del Mar Vernet M, Casamitjana M, et al. Trends in hormone therapy use before and after publication of the Women's Health Initiative trial: 10 years of follow-up. Menopause. 2009 Oct;16(5):1061–4.

58. Ettinger B, Wang SM, Leslie RS, Patel BV, Boulware MJ, Mann ME, et al. Evolution of postmenopausal hormone therapy between 2002 and 2009. Menopause. 2018 Nov;25(11):1306–12.

59. Grosse Y, Baan R, Straif K, Secretan B, Ghissassi FE, Bouvard V, et al. A review of human carcinogens—Part A: pharmaceuticals. Lancet Oncol. 2009 Jan;10(1):13–4.

60. Cogliano V, Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, et al. Carcinogenicity of combined oestrogen-progestagen contraceptives and menopausal treatment. Lancet Oncol. 2005 Aug;6(8):552–3.

61. Sumi C, Brinck-Johnsen T, Longnecker DS. Inhibition of a transplantable pancreatic carcinoma by castration and estradiol administration in rats. Cancer Res. 1989 Dec;49(23):6687–92.

62. Sumi C, Longnecker DS, Roebuck BD, Brinck-Johnsen T. Inhibitory effects of estrogen and castration on the early stage of pancreatic carcinogenesis in Fischer rats treated with azaserine. Cancer Res. 1989 May;49(9):2332–6.

63. Yakimchuk K, Iravani M, Hasni MS, Rhönnstad P, Nilsson S, Jondal M, et al. Effect of ligand-activated estrogen receptor β on lymphoma growth in vitro and in vivo. Leukemia. 2011 Jul;25(7):1103–10.

64. Shim G-J, Wang L, Andersson S, Nagy N, Kis LL, Zhang Q, et al. Disruption of the estrogen receptor beta gene in mice causes myeloproliferative disease resembling chronic myeloid leukemia with lymphoid blast crisis. Proc Natl Acad Sci U S A. 2003 May;100(11):6694–9.

65. Tanahashi NK, Suzawa N, Azuma C. Effects of sex hormones on oncogenesis in rat urinary bladder by N-butyl-N-(4-hydroxybutyl)-nitrosamine. Int J Clin Pharmacol Biopharm. 1977 Mar;15(3):101–5.

66. Johnson AM, O'Connell MJ, Messing EM, Reeder JE. Decreased bladder cancer growth in parous mice. Urology. 2008 Sep;72(3):470–3.

67. Yakimchuk K, Norin S, Kimby E, Hägglund H, Warner M, Gustafsson J-Å. Up-regulated estrogen receptor β2 in chronic lymphocytic leukemia. Leuk Lymphoma. 2012 Jan;53(1):139–44.

68. Shim G-J, Gherman D, Kim H-J, Omoto Y, Iwase H, Bouton D, et al. Differential expression of oestrogen receptors in human secondary lymphoid tissues. J Pathol. 2006 Feb;208(3):408–14.

69. Shen SS, Smith CL, Hsieh J-T, Yu J, Kim IY, Jian W, et al. Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue. Cancer. 2006 Jun;106(12):2610–6.

70. Blakeman PJ, Hilton P, Bulmer JN. Oestrogen and progesterone receptor expression in the female lower urinary tract, with reference to oestrogen status. BJU Int. 2000 Jul;86(1):32–8.

71. Cutolo M, Brizzolara R, Atzeni F, Capellino S, Straub RH, Puttini PCS. The immunomodulatory effects of estrogens: clinical relevance in immune-mediated rheumatic diseases. Ann N Y Acad Sci. 2010 Apr;1193:36–42.

72. Zen M, Ghirardello A, Iaccarino L, Tonon M, Campana C, Arienti S, et al. Hormones, immune response, and pregnancy in healthy women and SLE patients. Swiss Med Wkly. 2010 Apr;140(13–14):187–201.

73. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update. 2005 Aug;11(4):411–23.

74. Costas L, de Sanjosé S, Infante-Rivard C. Reproductive factors and non-Hodgkin lymphoma: a systematic review. Crit Rev Oncol Hematol. 2014 Dec;92(3):181–93.

75. Wahi MM, Shah N, Schrock CE, Rosemurgy AS 2nd, Goldin SB. Reproductive factors and risk of pancreatic cancer in women: a review of the literature. Ann Epidemiol. 2009 Feb;19(2):103–11.

76. Guan H-B, Wu L, Wu Q-J, Zhu J, Gong T. Parity and pancreatic cancer risk: a dose-response meta-analysis of epidemiologic studies. PloS One. 2014;9(3):e92738.

77. Zhu B, Zou L, Han J, Chen W, Shen N, Zhong R, et al. Parity and pancreatic cancer risk: evidence from a meta-analysis of twenty epidemiologic studies. Sci Rep. 2014 Jun;4:5313.

78. Tang B, Lv J, Li Y, Yuan S, Wang Z, He S. Relationship between female hormonal and menstrual factors and pancreatic cancer: a meta-analysis of observational studies. Medicine (Baltimore). 2015 Feb;94(7):e177.

79. Daugherty SE, Lacey JV, Pfeiffer RM, Park Y, Hoover RN, Silverman DT. Reproductive factors and menopausal hormone therapy and bladder cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2013 Jul;133(2):462–72.

80. Weibull CE, Eloranta S, Altman D, Johansson ALV, Lambe M. Childbearing and the risk of bladder cancer: a nationwide population-based cohort study. Eur Urol. 2013 Apr;63(4):733–8.

81. Kabat GC, Kim MY, Luo J, Hou L, Cetnar J, Wactawski-Wende J, et al. Menstrual and reproductive factors and exogenous hormone use and risk of transitional cell bladder cancer in postmenopausal women. Eur J Cancer Prev. 2013 Sep;22(5):409–16.

82. Davis-Dao CA, Henderson KD, Sullivan-Halley J, Ma H, West D, Xiang Y-B, et al. Lower risk in parous women suggests that hormonal factors are important in bladder cancer etiology. Cancer Epidemiol Biomarkers Prev. 2011 Jun;20(6):1156–70.

83. Dietrich K, Demidenko E, Schned A, Zens MS, Heaney J, Karagas MR. Parity, early menopause and the incidence of bladder cancer in women: a case-control study and meta-analysis. Eur J Cancer. 2011 Mar;47(4):592–9.

84. Huang A-T, Kogevinas M, Silverman DT, Malats N, Rothman N, Tardon A, et al. Bladder cancer and reproductive factors among women in Spain. Cancer Causes Control. 2009 Dec;20(10):1907–13.

85. Chavez-MacGregor M, Elias SG, Onland-Moret NC, van der Schouw YT, Van Gils CH, Monninkhof E, et al. Postmenopausal breast cancer risk and cumulative number of menstrual cycles. Cancer Epidemiol Biomarkers Prev. 2005 Apr;14(4):799–804.

86. Prizment AE, Anderson KE, Harlow BL, Folsom AR. Reproductive risk factors for incident bladder cancer: Iowa Women's Health Study. Int J Cancer. 2007 Mar;120(5):1093–8.

87. Pelucchi C, La Vecchia C, Negri E, Dal Maso L, Franceschi S. Smoking and other risk factors for bladder cancer in women. Prev Med. 2002 Aug;35(2):114–20.

88. Cantwell MM, Lacey JV, Schairer C, Schatzkin A, Michaud DS. Reproductive factors, exogenous hormone use and bladder cancer risk in a prospective study. Int J Cancer. 2006 Nov;119(10):2398–401.

89. Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. Int J Cancer. 2003 Jun;105(3):408–12.

90. Henderson BE, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis. 2000 Mar;21(3):427–33.

91. Lujan-Barroso L, Zhang W, Olson SH, Gao Y-T, Yu H, Baghurst PA, et al. Menstrual and Reproductive Factors, Hormone Use, and Risk of Pancreatic Cancer: Analysis From the International Pancreatic Cancer Case-Control Consortium (PanC4). Pancreas. 2016 Nov;45(10):1401–10.

92. Norman E Breslow, Nick E Day. Statistical methods in cancer research. The Analysis of Case-Controls Studies, Vol 1. Lyon, France: IARC; 1980.

93. Smith-Warner SA, Spiegelman D, Ritz J, Albanes D, Beeson WL, Bernstein L, et al. Methods for pooling results of epidemiologic studies: the Pooling Project of Prospective Studies of Diet and Cancer. Am J Epidemiol. 2006 Jun;163(11):1053–64.

94. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002 Jun;21(11):1539–58.

95. Galbraith RF. Graphical Display of Estimates Having Differing Standard Errors. Technometrics. 1988 Aug;30(3):271–81.

96. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002 Dec;5(6B):1113–24.

97. Buckland G, Travier N, Cottet V, González CA, Luján-Barroso L, Agudo A, et al. Adherence to the mediterranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. Int J Cancer. 2013 Jun;132(12):2918–27.

98. Klipstein-Grobusch K, Slimani N, Krogh V, Keil U, Boeing H, Overvad K, et al. Trends in self-reported past alcoholic beverage consumption and ethanol intake from 1950 to 1995 observed in eight European countries participating in the European Investigation into Cancer and Nutrition (EPIC). Public Health Nutr. 2002 Dec;5(6B):1297–310.

99. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2003 Jun;6(4):407–13.

100. Swerdlow SH. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 2008.

101. Wang M, Spiegelman D, Kuchiba A, Lochhead P, Kim S, Chan AT, et al. Statistical Methods for Studying Disease Subtype Heterogeneity. Stat Med. 2016 Feb;35(5):782–800.

102. Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. Eur J Endocrinol Eur Fed Endocr Soc. 2005 Apr;152(4):491–9.

103. Hayatbakhsh MR, Clavarino A, Williams GM, Sina M, Najman JM. Cigarette smoking and age of menopause: a large prospective study. Maturitas. 2012 Aug;72(4):346–52.

104. Ertunc D, Tok EC, Aytan H, Gozukara YM. Passive smoking is associated with lower age at menopause. Climacteric J Int Menopause Soc. 2015 Feb;18(1):47–52.

105. Tao X, Jiang A, Yin L, Li Y, Tao F, Hu H. Body mass index and age at natural menopause: a meta-analysis. Menopause. 2015 Apr;22(4):469–74.

106. Zhu D, Chung H-F, Pandeya N, Dobson AJ, Kuh D, Crawford SL, et al. Body mass index and age at natural menopause: an international pooled analysis of 11 prospective studies. Eur J Epidemiol. 2018 Aug;33(8):699–710.

107. Besson H, Ekelund U, Luan J, May AM, Sharp S, Travier N, et al. A cross-sectional analysis of physical activity and obesity indicators in European participants of the EPIC-PANACEA study. Int J Obes. 2009 Apr;33(4):497–506.

108. Dratva J, Gómez Real F, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause. 2009 Apr;16(2):385–94.

109. Gudmundsdottir SL, Flanders WD, Augestad LB. Physical activity and age at menopause: the Nord-Trøndelag population-based health study. Climacteric. 2013 Feb;16(1):78–87.

110. Emaus A, Dieli-Conwright C, Xu X, Lacey JV, Ingles SA, Reynolds P, et al. Increased long-term recreational physical activity is associated with older age at natural menopause among heavy smokers: the California Teachers Study. Menopause. 2013 Mar;20(3):282–90.

111. Zhao M, Whitcomb BW, Purdue-Smithe AC, Manson JE, Hankinson SE, Rosner BA, et al. Physical activity is not related to risk of early menopause in a large prospective study. Hum Reprod. 2018 01;33(10):1960–7.

112. Dunneram Y, Greenwood DC, Burley VJ, Cade JE. Dietary intake and age at natural menopause: results from the UK Women's Cohort Study. J Epidemiol Community Health. 2018 Aug;72(8):733–40.

113. Morris DH, Jones ME, Schoemaker MJ, McFadden E, Ashworth A, Swerdlow AJ. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. Am J Epidemiol. 2012 May 15;175(10):998–1005.

114. Whitcomb BW, Purdue-Smithe A, Hankinson SE, Manson JE, Rosner BA, Bertone-Johnson ER. Menstrual Cycle Characteristics in Adolescence and Early Adulthood Are Associated With Risk of Early Natural Menopause. J Clin Endocrinol Metab. 2018 Jan;103(10):3909–18.

115. Li J, Eriksson M, Czene K, Hall P, Rodriguez-Wallberg KA. Common diseases as determinants of menopausal age. Hum Reprod. 2016 Oct;

116. Forslund M, Landin-Wilhelmsen K, Schmidt J, Brännström M, Trimpou P, Dahlgren E. Higher menopausal age but no differences in parity in women with polycystic ovary syndrome compared with controls. Acta Obstet Gynecol Scand. 2019 Mar;98(3):320–6.

117. de Vries E, den Tonkelaar I, van Noord PA, van der Schouw YT, te Velde ER, Peeters PH. Oral contraceptive use in relation to age at menopause in the DOM cohort. Hum Reprod. 2001 Aug;16(8):1657–62.

118. Kaczmarek M. The timing of natural menopause in Poland and associated factors. Maturitas. 2007 Jun;57(2):139–53.

119. Zsakai A, Mascie-Taylor N, Bodzsar EB. Relationship between some indicators of reproductive history, body fatness and the menopausal transition in Hungarian women. J Physiol Anthropol. 2015;34:35.

120. Bjelland EK, Wilkosz P, Tanbo TG, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). Hum Reprod. 2014 Apr;29(4):835–41.

121. Kabat GC, Kamensky V, Rohan TE. Reproductive factors, exogenous hormone use, and risk of pancreatic cancer in postmenopausal women. Cancer Epidemiol. 2017 Aug;49:1–7.

122. Masoudi S, Momayez Sanat Z, Mahmud Saleh A, Nozari N, Ghamarzad N, Pourshams A. Menstrual and Reproductive Factors and Risk of Pancreatic Cancer in Women. Middle East J Dig Dis. 2017 Jul;9(3):146–9.

123. Andersson G, Borgquist S, Jirström K. Hormonal factors and pancreatic cancer risk in women: The Malmö Diet and Cancer Study. Int J Cancer. 2018;143(1):52–62.

124. Kane EV, Roman E, Becker N, Bernstein L, Boffetta P, Bracci PM, et al. Menstrual and reproductive factors, and hormonal contraception use: associations with non-Hodgkin lymphoma in a pooled analysis of InterLymph case-control studies. Ann Oncol. 2012 Sep;23(9):2362–74.

125. McGrath M, Michaud DS, De Vivo I. Hormonal and reproductive factors and the risk of bladder cancer in women. Am J Epidemiol. 2006 Feb;163(3):236–44.

126. Batra SC, Iosif CS. Progesterone receptors in the female lower urinary tract. J Urol. 1987 Nov;138(5):1301–4.

127. Duell EJ, Travier N, Lujan-Barroso L, Dossus L, Boutron-Ruault M-C, Clavel-Chapelon F, et al. Menstrual and reproductive factors in women, genetic variation in CYP17A1, and pancreatic cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. Int J Cancer. 2013 May;132(9):2164–75.

128. Lu Y, Wang SS, Sullivan-Halley J, Chang ET, Clarke CA, Henderson KD, et al. Oral contraceptives, menopausal hormone therapy use and risk of B-cell non-Hodgkin lymphoma in the California Teachers Study. Int J Cancer. 2011 Aug;129(4):974–82.

129. Wang SS, Voutsinas J, Chang ET, Clarke CA, Lu Y, Ma H, et al. Anthropometric, behavioral, and female reproductive factors and risk of multiple myeloma: a pooled analysis. Cancer Causes Control. 2013 Jul;24(7):1279–89.

130. Lee JS, Bracci PM, Holly EA. Non-Hodgkin lymphoma in women: reproductive factors and exogenous hormone use. Am J Epidemiol. 2008 Aug;168(3):278–88.

131. Lee E, Horn-Ross PL, Rull RP, Neuhausen SL, Anton-Culver H, Ursin G, et al. Reproductive factors, exogenous hormones, and pancreatic cancer risk in the CTS. Am J Epidemiol. 2013 Nov;178(9):1403–13.

132. Butt SA, Lidegaardi Ø, Skovlund C, Hannaford PC, Iversen L, Fielding S, et al. Hormonal contraceptive use and risk of pancreatic cancer-A cohort study among premenopausal women. PloS One. 2018;13(10):e0206358.

133. Morton LM, Wang SS, Richesson DA, Schatzkin A, Hollenbeck AR, Lacey JV. Reproductive factors, exogenous hormone use and risk of lymphoid neoplasms among women in the National Institutes of Health-AARP Diet and Health Study Cohort. Int J Cancer. 2009 Jun;124(11):2737–43.

134. Cerhan JR, Wallace RB, Folsom AR, Potter JD, Sellers TA, Zheng W, et al. Medical history risk factors for non-Hodgkin's lymphoma in older women. J Natl Cancer Inst. 1997 Feb;89(4):314–8.

135. Costas L, Casabonne D, Benavente Y, Becker N, Boffetta P, Brennan P, et al. Reproductive factors and lymphoid neoplasms in Europe: findings from the EpiLymph case-control study. Cancer Causes Control. 2012 Jan;23(1):195–206.

136. Prizment AE, Anderson KE, Hong C-P, Folsom AR. Pancreatic cancer incidence in relation to female reproductive factors: Iowa Women's Health Study. J Pancreas. 2007;8(1):16–27.

137. Navarro Silvera SA, Miller AB, Rohan TE. Hormonal and reproductive factors and pancreatic cancer risk: a prospective cohort study. Pancreas. 2005 May;30(4):369–74.

138. Skinner HG, Michaud DS, Colditz GA, Giovannucci EL, Stampfer MJ, Willett WC, et al. Parity, reproductive factors, and the risk of pancreatic cancer in women. Cancer Epidemiol Biomarkers Prev. 2003 May;12(5):433–8.

139. Lo A-C, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, et al. Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. Pancreas. 2007 Aug;35(2):120–9.

140. Nelson RA, Levine AM, Bernstein L. Reproductive factors and risk of intermediate- or high-grade B-Cell non-Hodgkin's lymphoma in women. J Clin Oncol. 2001 Mar;19(5):1381–7.

141. Beiderbeck AB, Holly EA, Sturkenboom MCJM, Coebergh JW, Stricker BHC, Leufkens HGM. No increased risk of non-Hodgkin's lymphoma with steroids, estrogens and psychotropics (Netherlands). Cancer Causes Control. 2003 Sep;14(7):639–44.

142. Mildon KH, Ansell P, Roman E, Kane EV. Reproductive factors, menopausal hormone therapy, and risk of non-Hodgkin, diffuse large B-cell and follicular lymphomas: a UK case-control study. Cancer Causes Control. 2010 Dec;21(12):2079–83.

143. Kane EV, Bernstein L, Bracci PM, Cerhan JR, Costas L, Dal Maso L, et al. Postmenopausal hormone therapy and non-Hodgkin lymphoma: a pooled analysis of InterLymph case-control studies. Ann Oncol. 2013 Feb;24(2):433–41.

144. Teras LR, Patel AV, Hildebrand JS, Gapstur SM. Postmenopausal unopposed estrogen and estrogen plus progestin use and risk of non-Hodgkin lymphoma in the American Cancer Society Cancer Prevention Study-II Cohort. Leuk Lymphoma. 2013 Apr;54(4):720–5.

145. Nørgaard M, Poulsen AH, Pedersen L, Gregersen H, Friis S, Ewertz M, et al. Use of postmenopausal hormone replacement therapy and risk of non-Hodgkin's lymphoma: a Danish population-based cohort study. Br J Cancer. 2006 May;94(9):1339–41.

146. Kato I, Chlebowski RT, Hou L, Wactawski-Wende J, Ray RM, Abrams J, et al. Menopausal estrogen therapy and non-Hodgkin's lymphoma: A post-hoc analysis of women's health initiative randomized clinical trial. Int J Cancer. 2016 Feb;138(3):604–11.

147. Humphrey LL, Chan BKS, Sox HC. Postmenopausal hormone replacement therapy and the primary prevention of cardiovascular disease. Ann Intern Med. 2002 Aug;137(4):273–84.

148. Lucas R, Azevedo A, Barros H. Self-reported data on reproductive variables were reliable among postmenopausal women. J Clin Epidemiol. 2008 Sep;61(9):945–50.

149. Colditz GA, Stampfer MJ, Willett WC, Stason WB, Rosner B, Hennekens CH, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. Am J Epidemiol. 1987 Aug;126(2):319–25.

150. den Tonkelaar I. Validity and reproducibility of self-reported age at menopause in women participating in the DOM-project. Maturitas. 1997 Jun;27(2):117–23.

151. Norell SE, Boethius G, Persson I. Oral contraceptive use: interview data versus pharmacy records. Int J Epidemiol. 1998 Dec;27(6):1033–7.

10. ANNEX

10.1. Annex 1: Ethical approval

Bellvitge Hospital Universitari
Institut Català de la Salut

Generalitat de Catalunya
Departament de Salut

INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA SOBRE PROYECTOS DE INVESTIGACIÓN

El Comité Ético de Investigación Clínica del Hospital Universitari de Bellvitge, mediante el procedimiento de evaluación rápida de la documentación contemplado en los Procedimientos Normalizados de Trabajo del Comité (esta aprobación constará en el Acta 11/16 de fecha 09/06/16), tras examinar toda la documentación presentada sobre el proyecto de Tesis Doctoral con nuestra ref. **PR171/16**, titulado:

"INFLUENCE OF MENSTRUAL FACTORS, REPRODUCTIVE HISTORY AND HORMONE USE ON WOMEN'S HELATH: AGE AT NATURAL MENOPAUSE, AND CANCERS OF THE PANCREAS, LUNG, AND BLADDER.",

De Leila Luján-Barroso (Dirigida por el Dr. Eric J. Duell) del Programa de Investigación en Epidemiología del Cáncer de la Unidad de Nutrición, Medio Ambiente y Cáncer del ICO, como DOCTORANDA, ha acordado emitir INFORME FAVORABLE al mencionado proyecto.

Que la composición actual del Comité Ético de Investigación Clínica es la siguiente:

Presidente	Dr. Francesc Esteve Urbano	Médico-Medicina Intensiva
Vicepresidente	Dra. Pilar Hereu Boher	Médico-Farmacología Clínica
Secretario	Dr. Enric Sospedra Martínez	Farmacia-Farmacia Hospitalaria
Vocales:	Dr. Josep Mª Arnau de Bolós	Médico-Farmacología Clínica
	Dra. María Berdasco Menéndez	Bióloga-miembro no sanitario
	Dr. Enric Condom Mundo	Médico-Anatomía Patológica
	Dr. Xavier Corbella Virós	Médico-Medicina Interna
	Sra. Consol Felip Farrás	Miembro laico-Docencia Investigación
	Dr. José Luis Ferreiro Gutiérrez	Médico-Cardiología
	Dra. Ana María Ferrer Artola	Farmacia-miembro sanitario
	Dr. Xavier Fulladosa Oliveras	Médico-Nefrología
	Dra. Margarita García Martín	Médico-Oncología Médica
	Dra. Laura Lladó Garriga	Médico-Cirugía General Digestiva
	Sra. Sonia López Ortega	Graduado Social-Atención Usuario
	Sra. Gemma Martínez Estalella	Enfermera-Enfermería
	Dr. Sergio Morchón Ramos	Medicina Preventiva
	Dr. Joan Josep Queralt Jiménez	Jurista
	Dr. Ricard Ramos Izquierdo	Medicina-Cirugía Torácica
	Dra. Gemma Rodríguez Palomar	Farmacia – Atención Primaria
	Dra. Nuria Sala Serra	Bióloga-miembro no sanitario
	Dr. Petru Cristian Simon	Médico-Farmacología Clínica

HUBC Hospital Universitari de Bellvitge
Feixa Llarga s/n
08907 L'Hospitalet de Llobregat
Tel. 932 607 500
www.bellvitgehospital.cat

Bellvitge Hospital Universitari
Institut Català de la Salut

Generalitat de Catalunya
Departament de Salut

Que este Comité cumple la legislación española vigente para este tipo de proyectos, así como las normas ICH y las Normas de Buena Práctica Clínica.

Lo que firmo en L'Hospitalet de Llobregat, a 24 de Mayo de 2016

Bellvitge Hospital Comitè Ètic d'Investigació Clínica

Fdo. Dr. Enric Sospedra Martínez
Secretario del CEIC

Hospital Universitari de Bellvitge
Feixa Llarga s/n
08907 L'Hospitalet de Llobregat
Tel. 932 607 500
www.bellvitgehospital.cat

10.2. Annex 2: Publication 1; Supplemental table

Table 8: Supplemental Table 1: Cox model for dose and duration of OC and menopause status in EPIC-Spain women

Dose and duration of OC[a]	Post-menopausal	HR (95%CI)[b]
Never used OC	773	Reference
Low dose of OC	262	0.96 (0.83- 1.12)
1-4 years high dose OC	79	0.74 (0.58- 0.93)
>4 years high dose OC	50	0.96 (0.71- 1.31)
Unknown	2	

[a] Where low dose of OC was considered if the year of first use was after 1972.

[b] HR > 1 indicates that the risk factor was associated with an earlier ANM and a HR <1 indicates that the risk factor was associated with a later ANM. Cox model stratified by center and age at recruitment and adjusted by smoking status, number of pregnancies, and time since regular menses.

10.3. Annex 3: Publication 3; web tables

Table 9: Web table 1: Baseline characteristics of women in the EPIC cohort, 1992-2000

	Ever parity[a]	High parity (3+ births)[a]	Ever oral contraceptives use[a]	Ever use of postmenopausal hormone therapy[a]	Hysterectomy + bilateral oophorectomy[a]
Age at recruitment (years), mean (range)	51.6(46.0-57.92)	53.60 (47.77-59.95)	48.70 (43.35-53.81)	54.95 (51.67-59.22)	57.32 (52.55- 62.24)
Age at diagnosis (years), mean (range)	64.13 (58.67-70.04)	64.69 (59.50-70.75)	62.11 (56.77-67.87)	65.09 (61.00-70.24)	68.27 (63.94- 71.61)
BMI, No. (%)					
<25 kg/m2	156138 (56.0)	43910 (49.5)	126227 (64.6)	47830 (59.3)	3686 (40.6)
25- <30 kg/m2	84727 (30.4)	29196 (32.9)	50858 (26.0)	24551 (30.4)	3390 (37.4)
≥30 kg/m2	38163 (13.7)	15658 (17.6)	18284 (9.4)	8290 (10.3)	1998 (22.0)
Physical activity, No. (%)					
Inactive	63355 (22.7)	23710 (26.7)	30924 (15.8)	15613 (19.4)	3172 (35.0)
Moderately inactive	93846 (33.6)	28621 (32.2)	66818 (34.2)	29298 (36.3)	3160 (34.8)
Moderately active	76450 (27.4)	23049 (26.0)	59580 (30.5)	22329 (27.7)	1625 (17.9)
Active	41218 (14.8)	12457 (14.0)	34554 (17.7)	12495 (15.5)	1074 (11.8)
Unknown	4159 (1.5)	927 (1.0)	3493 (1.8)	936 (1.2)	43 (0.5)
Educational level, No. (%)					
None	14333 (5.1)	8006 (9.0)	3770 (1.9)	1546 (1.9)	986 (10.9)
Primary school completed	73744 (26.4)	25952 (29.2)	34430 (17.6)	19281 (23.9)	3005 (33.1)
Technical/professional school	61835 (22.2)	16791 (18.9)	49083 (25.1)	20320 (25.2)	1656 (18.3)
Secondary school	63991 (22.9)	18788 (21.2)	48823 (25.0)	19615 (24.3)	1662 (18.3)
Longer education	54727 (19.6)	15460 (17.4)	52850 (27.1)	16082 (19.9)	1241 (13.7)
Not Specified	10398 (3.8)	3767 (4.2)	6413 (3.3)	3837 (4.7)	524 (5.8)
Smoking status, No. (%)					
Never	155256 (55.6)	53173 (59.9)	97466 (49.9)	42815 (53.1)	5744 (63.3)
Former	63616 (22.8)	18351 (20.7)	50920 (26.1)	20215 (25.1)	1808 (19.9)
Current	54219 (19.4)	15194 (17.1)	42891 (22.0)	15859 (19.7)	1389 (15.3)
Unknown	5937 (2.1)	2046 (2.3)	4092 (2.1)	1782 (2.2)	133 (1.5)
Country, No. (%)					
France	57587 (20.6)	18575 (20.9)	41788 (21.4)	21310 (26.4)	1707 (18.8)
Italy	27035 (9.7)	6526 (7.4)	12751 (6.5)	4602 (5.7)	1136 (12.5)
Spain	22455 (8.1)	10724 (12.1)	10679 (5.5)	2197 (2.7)	1217 (13.4)
UK	37316 (13.4)	11875 (13.4)	36481 (18.7)	11095 (13.8)	1551 (17.1)
The Netherlands	21480 (7.7)	6445 (7.3)	20021 (10.3)	4892 (6.1)	728 (8.0)
Greece	13923 (5.0)	4672 (5.3)	1482 (0.8)	672 (0.8)	783 (8.6)
Germany	23809 (8.5)	4648 (5.2)	22615 (11.6)	8345 (10.3)	793 (8.7)
Sweden	17890 (6.4)	5430 (6.1)	10536 (5.4)	4692 (5.8)	0 (0)
Denmark	25812 (9.3)	7974 (9.0)	16900 (8.7)	12715 (15.8)	1159 (12.8)
Norway	31721 (11.4)	11895 (13.4)	22116 (11.3)	10151 (12.6)	0 (0)
Alcohol consumption, No. (%)					
Never	24056 (8.6)	9271 (10.4)	10064 (5.2)	4219 (5.2)	1186 (13.1)
Former	11014 (4.0)	4357 (4.9)	5203 (2.7)	2436 (3.0)	694 (7.7)
Current	178304 (63.9)	54451 (61.3)	134003 (68.6)	56385 (69.9)	6581 (72.5)
Unknown	65654 (23.5)	20685 (23.3)	46099 (23.6)	17631 (21.9)	613 (6.8)

Abbreviations: BMI = body mass index; EPIC= European Prospective Investigation Into Cancer and Nutrition; NHL = non-Hodgkin lymphoma; p25–p75 = 25th–75th percentiles.

a) Numbers do not always add to the total because of missing values.

Table 10: Web table 2: Menstrual and reproductive characteristics collected at baseline (1992-2000) and risk of B-cell NHL in the EPIC cohort, by subtype

Characteristic	Person-years[c]	MM (n=387)		CLL/SLL (n=289)		DLBCL (n=302)		FL (n=264)	
		No. [c]	HR[d] (95%CI)	No. [c]	HR[d] (95%CI)	No. [c]	HR[d] (95%CI)	No. [c]	HR[d] (95%CI)
Age at menarche									
<12	699808	65	Reference	38	Reference	38	Reference	27	Reference
12	978186	77	0.84 (0.60- 1.17)	43	0.78 (0.50- 1.21)	46	0.83 (0.54- 1.27)	48	1.28 (0.80- 2.05)
13	1194471	94	0.79 (0.57- 1.08)	78	1.12 (0.76- 1.66)	87	1.23 (0.84- 1.81)	70	1.44 (0.92- 2.26)
14	1002365	78	0.70 (0.50- 0.98)	69	1.08 (0.72- 1.61)	62	0.96 (0.63- 1.44)	69	1.58 (1.01- 2.49)
>14	741051	65	0.68 (0.48- 0.97)	50	0.90 (0.58- 1.40)	55	1.00 (0.66- 1.54)	41	1.20 (0.73- 1.96)
P for trend			0.02		0.77		0.78		0.34
Full-term pregnancies									
Never	706477	46	Reference	34	Reference	36	Reference	39	Reference
1 birth	683853	54	0.91 (0.61- 1.36)	44	1.09 (0.69- 1.72)	41	0.97 (0.61- 1.53)	43	0.89 (0.57- 1.40)
2 births	1832221	137	0.86 (0.62- 1.21)	112	0.98 (0.66- 1.45)	108	0.91 (0.62- 1.34)	88	0.66 (0.44- 0.98)
3+ births	1244184	128	1.01 (0.72- 1.43)	86	0.95 (0.63- 1.43)	100	1.12 (0.76- 1.67)	83	0.90 (0.61- 1.35)
P for trend			0.88		0.60		0.49		0.54
Age at first full term pregnancy [a]									
<=20	563887	42	Reference	37	Reference	34	Reference	34	Reference
21- 23	1029538	86	1.09 (0.75- 1.59)	72	1.03 (0.69- 1.55)	55	0.90 (0.59- 1.40)	65	1.12 (0.73- 1.70)
24- 25	760262	77	1.28 (0.87- 1.88)	46	0.86 (0.55- 1.35)	64	1.39 (0.90- 2.14)	37	0.89 (0.55- 1.43)
26- 30	1112936	96	1.08 (0.74- 1.58)	59	0.73 (0.47- 1.13)	76	1.13 (0.74- 1.73)	61	1.00 (0.64- 1.55)
>30	392245	26	0.89 (0.53- 1.47)	30	1.09 (0.66- 1.81)	23	1.07 (0.62- 1.86)	20	0.94 (0.53- 1.68)
P for trend			0.80		0.37		0.38		0.64
Breastfeeding[a]									
Never	541260	37	Reference	25	Reference	30	Reference	28	Reference
Ever	3058525	262	1.10 (0.77- 1.57)	209	1.36 (0.89- 2.09)	209	1.10 (0.74- 1.64)	172	1.04 (0.69- 1.57)
Duration of breastfeeding[b]									
T1 (≤2 months)	976473	81	Reference	70	Reference	61	Reference	44	Reference
T2 (3- 8 months)	1005212	70	0.78 (0.56- 1.08)	74	0.94 (0.67- 1.31)	60	0.91 (0.63- 1.31)	65	1.40 (0.94- 2.07)
T3 (≥9 months)	1044614	111	1.13 (0.82- 1.54)	63	0.72 (0.50- 1.04)	85	1.31 (0.92- 1.86)	62	1.36 (0.89- 2.08)
P for trend			0.37		0.08		0.11		0.17

CI = confidence interval; CLL = chronic lymphocytic leukemia; DLBCL = diffuse ratio; MM = multiple myeloma; SLL = small lymphocytic lymphoma; T = Tertile.

a) Among parous women. // b) Among parous women who had ever breastfed. // c) Numbers do not always add to the total because of missing values. // d) All models stratified the data by center and age and adjusted the results for educational level.

Table 11: Web table 3: Exogenous hormone use and menopausal factors collected at baseline (1992–2000) and risk of B-cell NHL in the EPIC cohort, by subtype.

Characteristic	Person-years[b]	MM (n=387) No.[b]	MM (n=387) HR[c] (95%CI)	CLL/SLL (n=289) No.[b]	CLL/SLL (n=289) HR[c] (95%CI)	DLBCL (n=302) No.[b]	DLBCL (n=302) HR[c] (95%CI)	FL (n=264) No.[b]	FL (n=264) HR[c] (95%CI)
Oral contraceptives use									
Never	1919675	194	Reference	139	Reference	157	Reference	110	Reference
Ever	2731051	183	0.99 (0.79- 1.24)	142	0.97 (0.75- 1.27)	138	0.78 (0.61- 1.01)	148	0.98 (0.75- 1.29)
Duration of oral contraception									
Never	1919675	194	Reference	139	Reference	157	Reference	110	Reference
T1 (0.23- 3.9 years)	759811	48	0.91 (0.66-1.27)	51	1.21 (0.86-1.70)	36	0.71 (0.49-1.04)	49	1.16 (0.82-1.66)
T2 (4.0- 9.9 years)	879090	54	1.00 (0.72-1.38)	40	0.92 (0.63-1.35)	42	0.79 (0.55-1.14)	48	1.02 (0.71-1.47)
T3 (≥10 years)	833513	67	1.13 (0.83-1.54)	38	0.80 (0.54-1.19)	49	0.85 (0.60-1.21)	41	0.86 (0.58-1.27)
p-trend			0,50		0,24		0,37		0,52
Menopausal status									
Premenopausal	1693207	53	1.17 (0.70- 1.95)	52	1.67 (0.93- 2.99)	53	1.03 (0.58- 1.84)	61	1.16 (0.68- 1.96)
Perimenopausal	918876	66	1.04 (0.71- 1.53)	58	1.47 (0.96- 2.26)	41	0.71 (0.46- 1.09)	53	0.82 (0.56- 1.23)
Postmenopausal	2058663	250	Reference	166	Reference	190	Reference	140	Reference
Surgical postmenopuasal	121690	18	1.50 (0.93- 2.45)	13	1.48 (0.83- 2.62)	18	1.79 (1.10- 2.93)	10	1.33 (0.69- 2.55)
Age at natural menopause									
≤45	331008	36	Reference	29	Reference	30	Reference	24	Reference
>45- 50	661418	76	1.01 (0.68- 1.52)	54	0.90 (0.57- 1.42)	68	1.13 (0.73- 1.75)	47	1.01 (0.60- 1.68)
>50	595101	85	1.15 (0.77- 1.71)	53	0.87 (0.55- 1.37)	49	0.83 (0.53- 1.32)	38	0.94 (0.55- 1.60)
p-trend			0,43		0,49		0,32		0,73
Oophorectomy + Hysterectomy									
Neither	3253667	241	Reference	177	Reference	183	Reference	155	Reference
Oophorectomy	87407	6	0.77 (0.34-1.72)	5	0.89 (0.36- 2.16)	10	1.62 (0.86- 3.07)	6	1.19 (0.53- 2.71)
Hysterectomy	244685	22	0.86 (0.55- 1.34)	24	1.43 (0.93- 2.21)	25	1.29 (0.84- 1.97)	20	1.29 (0.80- 2.08)
Hysterectomy + oophorectomy	82879	28	1.27 (0.85- 1.89)	21	1.41 (0.89- 2.23)	19	1.18 (0.73- 1.91)	18	1.49 (0.91- 2.46)
Use of postmenopausal hormone therapy[a]									
No	1797159	186	Reference	132	Reference	149	Reference	105	Reference
Yes	11001200	115	1.04 (0.81- 1.34)	85	1.08 (0.80- 1.45)	88	0.95 (0.72- 1.26)	81	1.09 (0.80- 1.48)
Duration of postmenopausal hormone therapy									
Never	1797159	186	Reference	132	Reference	149	Reference	105	Reference
T1 (0.1- 1.25 years)	330183	28	0.90 (0.60- 1.35)	23	1.06 (0.67- 1.66)	25	0.93 (0.60- 1.43)	22	1.02 (0.64- 1.63)
T2 (1.33- 4.0 years)	345001	33	1.06 (0.72- 1.56)	22	0.98 (0.61- 1.57)	23	0.84 (0.53- 1.33)	28	1.18 (0.76- 1.82)

T3 (>4 years)	317805	39	1.09 (0.76- 1.60)	26	0.94 (0.60- 1.48)	33	1.04 (0.70- 1.55)	20	0.90 (0.55- 1.49)
p-trend			0,40		0,07		0,84		0,58
Type of postmenopausal hormone therapy[a]									
No	1797159	186	Reference	132	Reference	149	Reference	105	Reference
Estrogen alone	428936	32	0.93 (0.62- 1.39)	26	0.92 (0.57- 1.47)	29	0.87 (0.56- 1.36)	31	1.22 (0.78- 1.92)
Progestin alone	11837	2	NE	1	NE	1	NE	0	NE
Estrogen + Progestin	290446	39	0.95 (0.67- 1.35)	28	1.28 (0.82- 2.01)	24	0.96 (0.60- 1.53)	21	0.89 (0.54- 1.47)

CI = confidence interval; CLL = chronic lymphocytic leukemia; DLBCL = diffuse ratio; EPIC = European Prospective Investigation Into Cancer and Nutrition; HR= Hazard ratio; MM = multiple myeloma; NE= Not estimated; SLL = small lymphocytic lymphoma; T = Tertile. a) Among peri- and postmenopausal women (including surgical menopause).

b) Numbers do not always add to the total because of missing values.

c) All models stratified the data by center and age and adjusted the results for educational level.

Table 12: Web table 4: Menstrual and reproductive characteristic collected at baseline (1992-2000) and risk of B-cell NHL in the EPIC cohort (combining T and B subtypes and censoring multiple myeloma)

	Person-years	N	HR 95%CI	*P*-trend
Age at menarche, years				
<12	699808	138	Reference	0.46
12	978186	186	0.94 0.75, 1.17	
13	1194471	295	1.16 0.95, 1.43	
14	1002365	265	1.16 0.94, 1.43	
>14	741051	189	0.99 0.79, 1.24	
Missing		40		
Parity, no. of full-term pregnancies				
Nulliparous	706477	139	Reference	0.85
1	683853	163	0.98 0.78, 1.23	
2	1832221	411	0.88 0.72, 1.08	
3+	1244184	352	1.02 0.83, 1.25	
Missing		14		
Age at first full term pregnancy, years [a]				
<=20	563887	134	Reference	0.57
21- 23	1029538	251	1.03 0.83, 1.27	
24- 25	760262	187	1.03 0.82, 1.30	
26- 30	1112936	253	0.95 0.76, 1.19	
>30	392245	109	1.22 0.94, 1.59	
Missing		6		
Breastfeeding[1]				
Never	541260	107	Reference	
Ever	3058525	775	1.19 0.97, 1.47	
Missing		58		
Duration of breastfeeding [b]				
T1 (≤2 months)	976473	243	Reference	0.67
T2 (3- 8 months)	1005212	245	0.92 0.77, 1.11	
T3 (≥9 months)	1044614	281	1.04 0.86, 1.25	
Missing		6		

All models were adjusted by center, age and educational level. Numbers do not always add to the total because of missing values.Abbreviations: EPIC, European Prospective Investigation Into Cancer and Nutrition; HR = hazard ratio; T = Tertile; NHL = non-Hodgkin lymphoma.

a) Among parous women.

b) Among parous women who had ever breastfed.

Table 13: Web table 5: Exogenous hormone use and menopausal factors collected at baseline and risk of B-cell NHL in the EPIC cohort, 1992–1999 (combining T and B subtypes and censoring multiple myeloma)

	Person-years	N	HR	95%CI	*P*-trend
Oral contraceptives use					
Never	1919675	524	Reference		
Ever	2731051	565	0.92	0.80, 1.05	
Missing		24			
Duration of oral contraception					
Never	1919675	548	Reference		0.10
T1 (0.2- 3.9 years)	759811	182	1.02	0.86,1.22	
T2 (4.0- 9.9 years)	879090	167	0.89	0.74,1.07	
T3 (≥10.0 years)	833513	170	0.87	0.72,1.05	
Missing		46			
Menopausal status					
Premenopausal	1693207	218	1.20	0.90, 1.59	
Perimenopausal	918876	200	0.90	0.73, 1.10	
Postmenopausal (natural)	2058663	642	Reference		
Surgical postmenopausal	121690	53	1.54	1.16, 2.05	
Missing		0			
Age at natural menopause, years					
≤45	331008	102	Reference		0.41
>45- 50	661418	212	1.03	0.81, 1.31	
>50	595101	181	0.94	0.73, 1.20	
Missing		147			
Oophorectomy + Hysterectomy					
Neither	3253667	683	Reference		
Oophorectomy	87407	25	1.13	0.76, 1.69	
Hysterectomy	244685	85	1.25	0.99, 1.57	
Hysterectomy + oophorectomy	82879	75	1.33	1.04, 1.69	
Missing		245			
Use of postmenopausal hormone therapy [a]					
Never	1797159	507	Reference		
Ever	11001200	331	1.01	0.87, 1.17	
Missing		57			
Duration of postmenopausal hormone therapy					
Never	1797159	507	Reference		0.60
T1 (0.1- 1.25 years)	330183	109	1.01	0.81, 1.26	
T2 (1.26- 4.0 years)	345001	98	0.97	0.77, 1.21	
T3 (>4 years)	317805	121	0.94	0.75, 1.18	
Missing		57			
Type of postmenopausal hormone therapy [a]					
Never	1797159	507	Reference		
Estrogen alone	428936	109	0.94	0.74, 1.18	
Progestin alone	11837	3	NE		
Estrogen + Progestin	290446	98	1.03	0.81, 1.30	
Missing		121			

All models were stratified by center and age and adjusted by educational level. Numbers do not always add to the total because of missing values. Abbreviations: EPIC, European Prospective Investigation Into Cancer and Nutrition; HR = hazard ratio; T = Tertile; NHL = non-Hodgkin lymphoma; NE= Not estimated

a) Among peri- and postmenopausal women (including surgical menopause).

10.4. Annex 4: Publication 4; Supplemental table

Table 14: Supplemental Table 1: Baseline characteristics of women in the EPIC cohort by country

	Cohort (n= 333 919)	France (n= 67 403)	Italy (n= 30 513)	Spain (n= 24 850)	United Kingdom (n= 52 566)	The Netherlands (n= 26 912)	Greece (n= 15 233)	Germany (n= 27 379)	Sweden (n= 26 368)	Denmark (n= 28 720)	Norway (n= 33 975)
Urothelial Carcinoma cases	529	40	72	32	68	80	7	25	105	80	20
Age at recruitment(years)[a]	51 (45- 58)	51 (47- 57)	51 (44- 57)	48 (41- 55)	48 (36- 58)	53 (46- 59)	54 (43- 64)	48 (41- 57)	51 (47- 60)	56 (53- 60)	48 (44- 52)
Age at diagnosis(years)[a]	68 (62- 74)	65 (60- 71)	65 (59- 71)	64 (57- 71)	63 (52- 73)	67 (59- 73)	65 (54- 75)	59 (52- 67)	69 (60- 78)	72 (68- 76)	61 (58- 65)
BMI(kg/m^2)[a]	24.1 (21.9- 27.2)	22.5 (20.8- 24.7)	25.0 (22.6- 27.9)	27.5 (24.7- 30.9)	23.4 (21.4- 26.1)	24.5 (22.3- 27.3)	28.2 (24. 8- 31.6)	24.7 (22.3- 28.0)	24.1 (21. 9- 27.0)	24.8 (22.5- 27.8)	23.8 (21.8- 26.2)
Physical activity [b]											
Inactive	73 114 (21.9)	12 623 (18.7)	11 201 (36.7)	12 071 (48.6)	12 581 (23.9)	1 897 (7.1)	8 157 (53.6)	4 756 (17.4)	5 532 (21.0)	3 050 (10.6)	1 246 (3.7)
Moderately inactive	113 292 (33.9)	26 969 (40.0)	11 940 (39.1)	8 745 (35.2)	18 867 (35.9)	6 410 (23.8)	3 997 (26.2)	10 378 (37.9)	9 480 (36.0)	9 235 (32.2)	7 271 (21.4)
Moderately active	90 980 (27.3)	21 813 (32.4)	4 557 (14.9)	2 983 (12.0)	12 075 (23.0)	6 480 (24.1)	2 460 (16.2)	7 110 (26.0)	6 912 (26.2)	7 148 (24.9)	19 442 (57.2)
Active	50 782 (15.2)	5 998 (8.9)	2 815 (9.2)	1 051 (4.2)	8 056 (15.3)	9 399 (34.9)	619 (4.1)	5 129 (18.7)	4 400 (16.7)	9 265 (32.3)	4 050 (11.9)
Unknown	5 751 (1.7)				987 (1.9)	2 726 (10.1)		6 (0.02)	44 (0.2)	22 (0.1)	1 966 (5.8)
Smoking status [b]											
Never	186 228 (55.8)	44 938 (66.7)	16 376 (53.7)	17 740 (71.4)	31 544 (60.0)	10 984 (40.8)	11 144 (73.2)	15 333 (56.0)	13 957 (52.9)	12 563 (43.7)	11 649 (34.3)
Former	75 216 (22.5)	12 896 (19.1)	6 162 (20.2)	2 446 (9.8)	14 457 (27.5)	8 425 (31.3)	816 (5.4)	7 017 (25.6)	6 004 (22.8)	7 074 (24.6)	9 919 (29.2)
Current	64 756 (19.4)	5 807 (8.6)	7 974 (26.1)	4 652 (18.7)	5 543 (10.5)	7 409 (27.5)	2 594 (17.0)	4 980 (18.2)	6 282 (23.8)	9 021 (31.4)	10 494 (30.9)
Unknown	7 719 (2.3)	3 762 (5.6)	1 (0.0)	12 (0.1)	1 022 (1.9)	94 (0.4)	679 (4.5)	49 (0.2)	125 (0.5)	62 (0.2)	1 913 (5.6)
Smoking status and intensity [b]											
Never	161 061 (48.2)	25 164 (37.3)	12 657 (41.5)	17 740 (71.4)	31 544 (60.0)	10 938 (40.6)	1 1101 (72.9)	15 333 (56.0)	12 436 (47.2)	12 563 (43.7)	11 585 (34.1)
Current ≤15 cigarettes/day	40 802 (12.2)	2 971 (4.4)	4 611 (15.1)	2 950 (11.9)	3 675 (7.0)	4 435 (16.5)	1 425 (9.4)	3 491 (12.8)	4 482 (17.0)	5 978 (20.8)	6 784 (20.0)
Current >15 cigarettes/day	21 318	1 924	3 360	1 660	1 409	2 540	1 162	1 467	1 512	2 954	3 330

	(6.4)	(2.9)	(11.0)	(6.7)	(2.7)	(9.4)	(7.6)	(5.4)	(5.7)	(10.3)	(9.8)
Former quit ≤ 10 years	27 394 (8.2)	3 628 (5.4)	2 959 (9.7)	1 473 (5.9)	4 887 (9.3)	3 011 (11.2)	478 (3.1)	2 363 (8.6)	2 349 (8.9)	2 322 (8.1)	3 924 (11.6)
Former quit >10 years	44 918 (13.5)	8 581 (12.7)	3 188 (10.5)	936 (3.8)	8 977 (17.1)	5 215 (19.4)	298 (2.0)	4 361 (15.9)	3 482 (13.2)	4 268 (14.9)	5 612(16.5)
Current, pipe/cigar/occasional cigarette smokers	27 610 (8.3)	21 818 (32.4)	3 719 (12.2)	13 (0.1)	145 (0.3)	46 (0.2)	44 (0.3)	21 (0.1)	1 672 (6.3)	68 (0.2)	64 (0.2)
Current/Former, missing	4 854 (1.5)	1 312 (2.0)	18 (0.1)	66 (0.3)	907 (1.7)	633 (2.4)	46 (0.3)	294 (1.1)	310 (1.2)	505 (1.8)	763 (2.3)
Unknown	5 962 (1.8)	2 005 (3.0)	1 (0.0)	12(0.1)	1022 (1.9)	94 (0.4)	679 (4.5)	49 (0.2)	125 (0.5)	62 (0.2)	1 913 (5.6)
Vegetables intake(g/day)[a]	186 (118-286)	264 (189-356)	162 (109-232)	216 (138-315)	256 (186-347)	127 (98-162)	412 (317-527)	117 (89-156)	119 (70-184)	172 (112-244)	126 (87-179)
Fruit intake(g/day)[a]	216 (125-332)	242 (153-339)	320 (221-443)	286 (176-436)	229 (143-345)	195 (123-288)	344 (244-457)	126 (92-204)	179 (114-269)	172 (100-276)	138 (79-219)
Job exposure [b, c, d]											
No	100 681 (93.6)			23 673 (95.3)	10 971 (94.8)		14 730 (96.9)	24 900 (91.0)		26 407 (92.3)	
Yes	6 920 (6.4)			1 177 (4.7)	599 (5.2)		465 (3.1)	2 479 (9.1)		2 200 (7.7)	
Diabetes [b]											
No	300 864 (97.3)	65 960 (98.0)	29 846 (97.9)	23 681 (95.5)	35 647 (98.3)	26 229 (97.8)	14 182 (93.3)	26 590 (97.1)	24 437 (98.2)	27 117 (94.8)	27 175 (98.5)
Yes	7 422 (2.4)	1 379 (2.1)	633 (2.1)	1 124 (4.5)	633 (1.7)	581 (2.2)	1 016 (6.7)	775 (2.8)	445 (1.8)	430 (1.5)	406 (1.5)
Do not known	1 078 (0.4)							8 (0.03)		1 070 (3.7)	

UC: Urothelial Carcinoma // BMI: Body mass index

[a] Median (percentile 25th and percentile 75th)

[b] n (%)

[c] Available in Spain, Cambridge, Greece, Germany, and Denmark, Germany.

[d] Job exposure was coded as “yes” if the participant worked exposure to heavy metals, aromatic amines, polycyclic aromatic hydrocarbons, and environmental tobacco smoking.

Table 15: Supplemental Table 2: Reproductive factors, menstrual, menopausal factors, and exogenous hormone use in relation to UC by aggressiveness and by grade in EPIC Women.

	Nonaggressive (n=146)		Aggressive (n=230)		Low-Grade (n=80)		High-Grade (n=233)	
	Cases (%)	HR (95%CI) [a]	Cases (%)	HR (95%CI) [a]	Cases (%)	HR (95%CI) [a]	Cases (%)	HR (95%CI) [a]
Age at menarche, years								
<12	12 (8.4)	1.00 (referent)	33 (14.4)	1.00 (referent)	10(12.5)	1.00 (referent)	25(10.7)	1.00 (referent)
12	26 (17.8)	1.39 (0.70- 2.76)	45 (19.6)	0.96 (0.61- 1.51)	7(8.8)	0.47(0.18-1.24)	51(21.9)	1.41(0.87-2.29)
13	37 (25.3)	1.64 (0.85- 3.17)	55 (23.9)	0.91 (0.59- 1.41)	23(28.8)	1.29(0.61-2.75)	60(25.8)	1.36(0.85-2.19)
14	36 (24.7)	1.74 (0.90- 3.39)	45 (19.6)	0.74 (0.47- 1.18)	20(25.0)	1.26(0.58-2.76)	50(21.5)	1.23(0.75-2.00)
>14	32 (21.9)	1.80 (0.91- 3.57)	47 (20.4)	0.81 (0.51- 1.29)	19(23.8)	1.46(0.65-3.24)	41(17.6)	1.13(0.68-1.89)
Unknown	3 (2.1)		5 (2.2)		1 (1.3)		6 (2.6)	
***P*-trend**		0.075		0.188		0.057		0.903
Cumulative duration of menstrual cycling, accounting for OC use, years [b]								
<23	17 (11.6)	1.00 (referent)	29 (12.6)	1.00 (referent)	9(11.3)	1.00 (referent)	28(12.0)	1.00 (referent)
23- <30	31 (21.2)	1.29 (0.70- 2.36)	41 (17.8)	1.09 (0.67- 1.78)	18(22.5)	1.59(0.69-3.65)	44(18.9)	0.98(0.60-1.59)
30- <35	32 (21.9)	1.14 (0.62- 2.12)	47 (20.4)	0.94 (0.58- 1.53)	19(23.8)	1.48(0.63-3.46)	42(18.0)	0.74(0.45-1.22)
≥35	37 (25.3)	1.14 (0.61- 2.12)	63 (27.4)	1.17 (0.73- 1.87)	21(26.2)	1.57(0.66-3.71)	65(27.9)	0.99(0.61-1.61)
Unknown	29 (18.9)	1.19 (0.60-2.35)	50 (21.7)	1.01 (0.61- 1.67)	13 (16.3)	1.53 (0.59-3.98)	54 (23.2)	1.01 (0.60- 1.71)
***P*-trend**		0.396		0.610		0.348		0.982
Use of OC								
No	80 (54.8)	1.00 (referent)	123 (53.5)	1.00 (referent)	38(47.5)	1.00 (referent)	137(58.8)	1.00 (referent)
Yes	65 (44.5)	0.79 (0.54- 1.15)	103 (44.8)	0.90 (0.67- 1.21)	42(52.5)	0.98(0.59-1.63)	94(40.3)	0.80(0.59-1.08)
Unknown	1 (0.7)		4 (1.7)				2(0.9)	
Duration OC use, years								
No	80 (54.8)	1.00 (referent)	123 (53.5)	1.00 (referent)	38 (47.5)	1.00 (referent)	137 (58.8)	1.00 (referent)

>0- ≤1	6 (4.1)	0.40 (0.17- 0.82)	19 (8.3)	0.84 (0.51- 1.39)	5 (6.3)	0.65 (0.25- 1.70)	14 (6.0)	0.57 (0.32-1.00)
>1- 5	16 (11.0)	0.79 (0.45- 1.40)	24 (10.4)	0.85 (0.54- 1.35)	10 (12.5)	0.94 (0.45- 1.98)	19 (8.2)	0.65 (0.39- 1.07)
>5- 10	19 (13.0)	1.03 (0.60- 1.78)	28 (12.2)	1.12 (0.72- 1.74)	15 (18.8)	1.53 (0.79- 2.99)	25 (10.7)	0.96 (0.61- 1.52)
>10	17 (11.6)	0.86 (0.48- 1.53)	22 (9.6)	0.74 (0.46- 1.21)	6 (7.5)	0.41 (0.20- 1.31)	25 (10.7)	0.93 (0.58- 1.50)
Unknown duration	7 (4.8)		10 (4.4)		6 (7.5)		11 (4.7)	
Unknown use of OC	1 (0.7)		4 (1.7)				2 (0.9)	
***P* trend**		0.769		0.469		0.712		0.549
Menopausal status								
Premenopausal	18 (12.3)	1.00 (referent)	15 (6.5)	1.00 (referent)	12(15.0)	1.00 (referent)	23(9.9)	1.00 (referent)
Perimenopausal	21 (14.4)	0.87 (0.37- 2.04)	22 (9.6)	1.64 (0.67- 4.00)	15(18.8)	1.19(0.39-3.58)	25(10.7)	1.56(0.71-3.43)
Natural postmenopausal	102 (69.9)	1.26 (0.52- 3.02)	180 (78.3)	2.47 (1.01- 6.03)	51(63.8)	1.16(0.35-3.81)	175(75.1)	1.60(0.60-4.22)
Surgical postmenopuasal	5 (3.4)	1.11 (0.33- 3.75)	13 (5.7)	3.25 (1.18- 8.97)	2(2.5)	0.80(0.13-4.81)	10(4.3)	1.08(0.50-2.36)
Age at natural menopause, years [c]								
≤46	21 (20.6)	1.14 (0.64- 2.05	39 (21.7)	1.14 (0.73- 1.76)	8(15.7)	0.84 (0.35- 2.02)	39(22.3)	1.16 (0.75- 1.79)
47- 49	23 (22.6)	1.40 (0.79- 2.47)	28 (15.6)	1.00 (0.62- 1.63)	12(23.5)	1.32 (0.60- 2.89)	25(14.3)	0.87 (0.53- 1.43)
50 - 52	26 (25.5)	1.00 (referent)	43 (23.9)	1.00 (referent)	14(27.5)	1.00 (referent)	45(25.7)	1.00 (referent)
≥53	16 (15.7)	1.01 (0.54- 1.91)	40 (22.2)	1.49 (0.96- 2.31)	10(19.6)	1.21 (0.52- 2.79)	36(20.6)	1.35 (0.86- 2.10)
Unknown	16 (15.7)	1.26 (0.63- 2.51)	30 (16.7)	1.18 (0.72- 1.95)	7 (13.7)	1.11 (0.41- 3.06)	30(17.1)	1.26 (0.76- 2.09)
***P*-trend**		0.688		0.324		0.53		0.571
Age at menopause, years								
≤46	24 (22.4)	1.14 (0.65- 2.0)	49 (25.4)	1.19 (0.79- 1.80)	9 (17.0)	0.83 (0.36- 1.96)	47 (25.4)	1.17 (0.76- 1.76)
47- 49	24 (22.4)	1.37 (0.78- 2.38)	28 (14.5)	0.92 (0.57- 1.47)	13 (24.5)	1.37 (0.64- 2.95)	25 (13.5)	0.82 (0.50- 1.34)
50 - 52	27 (25.2)	1.00 (referent)	46 (23.8)	1.00 (referent)	14 (26.4)	1.00 (referent)	47 (25.4)	1.00 (referent)
≥53	16 (15.0)	0.98 (0.52- 1.83)	40 (20.7)	1.43 (0.93- 2.20)	10 (18.9)	1.21 (0.53- 2.79)	36 (19.5)	1.30 (0.83- 2.02)
Unknown	16 (15.0)	1.31 (0.66- 2.60)	30 (15.5)	1.11 (0.68- 1.82)	7 (13.2)	1.20 (0.44- 3.29)	30 (16.2)	1.24 (0.75- 2.05)
***P*-trend**		0.635		0.479		0.532		0.681
Use of MHT [d]								

No	60 (46.9)	1.00 (referent)	122 (56.7)	1.00 (referent)	28(41.2)	1.00 (referent)	124(62.9)	1.00 (referent)
Yes	53 (41.4)	1.93 (1.29- 2.87)	85 (39.5)	1.27 (0.94- 1.71)	31(45.6)	2.37(1.37-4.12)	73(37.1)	1.33(0.97-1.82)
Unknown	15 (11.7)	1.72 (0.76-3.87)	8 (3.7)		9(13.2)	2.93 (0.94- 9.11)	13(6.2)	
Duration MHT use, years [d]								
No	60 (46.9)	1.00 (referent)	122 (56.7)	1.00 (referent)	28 (41.2)	1.00 (referent)	124 (59.1)	1.00 (referent)
≤1.25	19 (14.8)	2.31 (1.35- 3.94)	22 (10.2)	1.11 (0.70-1. 77)	15(22.1)	3.77 (1.95- 7.31)	19(9.1)	1.10 (0.67-1.80)
>1.25-4	12 (9.4)	1.47 (0.77- 2.80)	27 (12.6)	1.60 (1.03- 2.48)	9(13.2)	2.28 (1.03- 5.04)	18(8.6)	1.16 (0.69- 1.94)
>4	17 (13.3)	2.32 (1.29- 4.17)	29 (13.5)	1.11 (0.72- 1.72)	6(8.8)	1.79 (0.70- 4.60)	24(11.4)	1.48 (0.92- 2.38)
Unknown duration	5 (3.9)		7 (3.3)		1(1.5)		12 (5.7)	
Unknown use of MHT	15 (11.7)	1.56 (0.67- 3.61)	8 (3.7)		9(13.2)	2.26 (0.68- 7.49)	13 (6.2)	
***P*-trend**		0.002		0.242		0.023		0.100
Type of MHT [d, e]								
Non-users of MHT	55 (53.4)	1.00 (referent)	111 (58.4)	1.00 (referent)	26(48.2)	1.00 (referent)	114(64.0)	1.00 (referent)
Oestrogen alone	7 (6.8)	1.47 (0.65- 3.30)	19 (10.0)	1.59 (0.96- 2.64)	5(9.3)	2.59 (0.97- 6.95)	13(7.3)	1.26(0.69-2.28)
Oestrogen + Progestin	17 (23.3)	1.57 (0.84- 2.94)	22 (11.6)	0.92 (0.56- 1.50)	9(16.7)	1.59 (0.67-3.77)	23(12.9)	1.09(0.65-1.80)
Unknown type	24 (23.3)	2.37 (1.44- 3.91)	38 (20.0)	1.16 (0.79- 1.70)	14(25.9)	2.76 (1.40- 5.46)	28(15.7)	1.23 (0.80- 1.87)
Oophorectomy [f]								
No	102 (81.0)		171 (77.4)	1.00 (referent)	56(82.4)		170(78.7)	1.00 (referent)
Unilateral	5 (4.0)		16 (7.2)	1.51 (0.90- 2.52)	3(4.4)		11(5.1)	1.06(0.57-1.95)
Bilateral	5 (4.0)		14 (6.3)	1.36 (0.78- 2.36)	2(2.9)		11(5.1)	1.04(0.56-1.94)
Unknown if unilateral or bilateral	0 (0)		1 (0.5)		19(10.3)		24 (11.1)	0.85 (0.31- 2.28)
Unknown	14 (11.1)		19 (8.6)					
Hysterectomy [f]								
No	99 (78.6)	1.00 (referent)	169 (76.5)	1.00 (referent)	55(80.5)	1.00 (referent)	166(78.7)	1.00 (referent)
Yes	20 (15.9)	0.96 (0.59 1.57)	38 (17.2)	1.11 (0.78- 1.59)	11(16.2)	1.03(0.53-1.99)	37(17.1)	1.06(0.73-1.52)
Unknown	7 (5.6)		14 (6.3)		2 (2.9)		13(6.0)	
Parity								
No	27 (18.5)	1.00 (referent)	29 (12.6)	1.00 (referent)	18(22.5)	1.00 (referent)	29(12.5)	1.00 (referent)
Yes	115 (78.8)	0.59 (0.39- 0.90)	196 (85.2)	0.91 (0.62- 1.35)	59(73.8)	0.44(0.26-0.75)	199(85.4)	0.96(0.65-1.43)

Unknown	4 (2.7)		5 (2.2)		3(3.8)		5(2.2)	
Number of full-term pregnancies [g]								
0 [h]	26 (18.7)	1.42 (0.81- 2.51)	26 (11.9)	0.79 (0.48- 1.29)	18(23.1)	1.70 (0.83- 3.46)	25(11.5)	0.80 (0.48-1.33)
1	23 (16.5)	1.00 (referent)	43 (19.6)	1.00 (referent)	14(18.0)	1.00 (referent)	39(18.0)	1.00 (referent)
2	43 (30.9)	0.71 (0.42- 1.19)	89 (40.6)	0.81 (0.56- 1.17)	24(30.8)	0.65 (0.33-1.28)	77(35.5)	0.78(0.53-1.16)
≥3	43 (30.9)	0.83 (0.49- 1.41)	56 (25.6)	0.59 (0.39- 0.90)	19(24.4)	0.63 (0.30-1.29)	71(32.7)	0.81(0.53-1.21)
Unknown	4 (2.9)		5 (2.3)		3(3.9)		5 (2.3)	
***P*-trend** [i]		0.039		0.067		0.002		0.674
Age at first full term pregnancy, years [j]								
≤20	15 (13.0)	1.00 (referent)	33 (16.8)	1.00 (referent)	12 (20.3)	1.00 (referent)	28 (14.1)	1.00 (referent)
21- 23	30 (26.1)	0.98 (0.52- 1.83)	57 (29.1)	1.09 (0.70- 1.68)	13 (22.0)	0.57 (0.26-1.26)	49 (24.6)	0.84 (0.53-1.35)
24- 25	21 (18.3)	0.83 (0.42- 1.64)	33 (16.8)	0.88 (0.53- 1.44)	9 (15.3)	0.51 (0.21-1.25)	38 (19.1)	0.81 (0.49-1.35)
26- 30	38 (33.0)	0.94 (0.50- 1.74)	55 (28.1)	0.96 (0.61- 1.52)	22 (37.3)	0.79 (0.37-1.65)	60 (30.2)	0.80 (0.50-1.27)
≥30	11 (9.6)	0.85 (0.38- 1.88)	17 (8.7)	0.96 (0.53- 1.76)	3 (5.1)	0.33 (0.09-1.22)	23 (11.6)	0.95 (0.54-1.68)
Unknown			1 (0.5)				1(0.5)	
***P*-trend**		0.702		0.661		0.402		0.713
Breastfeeding [i, j]								
No	19 (18.1)	1.00 (referent)	24 (13.4)	1.00 (referent)	11 (20.0)	1.00 (referent)	32 (17.8)	1.00 (referent)
Yes	83 (79.1)	0.82 (0.49- 1.36)	155 (86.6)	0.97 (0.62- 1.51)	43 (78.2)	0.66 (0.33-1.32)	146 (81.1)	0.83 (0.56-1.24)
Unknown	3 (2.9)				1(1.8)		2 (1.1)	
Duration of breastfeeding, all pregnancies, months [j, k]								
>0-≤3	26 (31.3)	1.00 (referent)	53 (34.2)	1.00 (referent)	14 (32.6)	1.00 (referent)	46 (31.5)	1.00 (referent)
>3- 12	39 (47.0)	0.98 (0.58- 1.66)	66 (42.6)	0.75 (0.51- 1.11)	16 (37.2)	0.83 (0.39-1.76)	68 (46.6)	0.93 (0.63-1.39)
>12	18 (21.7)	0.82 (0.41- 1.65)	33 (21.3)	0.75 (0.45- 1.24)	13 (30.2)	1.42 (0.60-3.34)	31 (21.2)	0.69 (0.40-1.16)
Unknown			3 (1.9)				1 (0.7)	
***P*-trend**		0.600		0.234		0.388		0.219
Induced abortions [l]								
Never pregnant	17 (15.9)	1.70 (1.00- 2.91)	19 (9.8)	1.01 (0.63- 1.64)	13(21.7)	2.66 (1.40- 5.07)	16(9.0)	0.83 (0.49- 1.40)
0	69 (64.5)	1.00 (referent)	137 (70.6)	1.00 (referent)	35(58.3)	1.00 (referent)	134(74.4)	1.00 (referent)
1	14 (14.0)	1.90 (1.05- 3.42)	25 (12.9)	1.04 (0.67- 1.62)	9(15.0)	1.67 (0.77- 3.61)	18(10.0)	1.22 (0.73- 2.04)
≥2	5 (3.5)	1.22 (0.47- 3.16)	11 (5.7)	1.00 (0.53- 1.90)	2(3.3)	0.67 (0.16- 2.91)	10(5.6)	1.19 (0.60- 2.36)
Unknown	1 (0.9)		2 (1.0)		1(1.7)		2(1.1)	

***P*-trend**		0.657		0.947		0.119		0.261
Spontaneous abortions [m]								
Never pregnant	22 (17.3)	1.77 (1.10- 2.86)	19 (9.4)	0.95 (0.59- 1.55)	17(23.6)	2.83 (1.59- 5.03)	17(8.6)	0.80(0.48-1.34)
0	76 (59.8)	1.00 (referent)	135 (66.5)	1.00 (referent)	40(55.6)	1.00 (referent)	128(65.0)	1.00 (referent)
1	21 (16.5)	1.15 (0.71- 1.86)	33 (16.3)	1.01 (0.69- 1.48)	10(13.9)	1.05 (0.53- 2.11)	35(17.8)	1.13(0.78-1.65)
≥2	7 (5.5)	0.96 (0.44- 2.09)	14 (6.9)	1.25 (0.72- 2.17)	4(5.6)	1.16 (0.41- 3.24)	15(7.6)	1.26(0.72-2.15)
Unknown	1 (0.8)		2 (1.0)		1(1.4)		2 (1.0)	
***P*-trend**		0.225		0.710		0.048		0.164
Fertility problems [n]								
No	82 (73.2)		107 (77.5)		45(75.0)	-	142(75.5)	
Yes	7 (6.3)		4 (2.9)		2(3.3)	-	8(4.3)	
Missing	23 (20.5)		27 (19.6)		13 (21.7)		38(20.2)	

OC: oral contraceptive // MHT: menopause hormone therapy

[a]Cox proportional hazards model stratified by center and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b]Cox proportional hazards model stratified by center and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake, OC use, and full-term pregnancies.

[c] Women who had surgical menopause were excluded

[d] In peri and postmenopausal women (natural or surgical).

[e] Available in France, Italy, Spain, United kingdom, The Netherlands, Germany, Denmark, and Norway.

[f] Available in all centers except Malmö.

[g] Available in all centers excepte Bilthoven.

[h] Including nulliparous women and women without full-term pregnancies.

[i] In parous women.

[j] Available in all centers except Bilthoven and Umeå**.**

[k] In parous women who has ever breastfed.

[l] Available in all centers excepte Bilthoven, Umeå, Malmö, and Norway

[m] Available in all centers excepte Bilthoven, Umeå, and Norway.

[n] Available in France, Italy, Spain, United Kingdom, Utrecht, Greece, and Germany.

Table 16: Supplemental table 3: Reproductive factors, menstrual, menopausal factors, and exogenous hormone use in relation to UC by smoking status in EPIC Women.

	Never		Former		Current	
	Cases (%) n =195	HR (95%CI)[a]	Cases (%) n=133	HR (95%CI)[b]	Cases (%) n=197	HR (95%CI)[b]
Age at menarche, years						
<12	25 (12.8)	1.00 (referent)	13 (9.8)	1.00 (referent)	26 (13.2)	1.00 (referent)
12	35 (18.0)	0.95 (0.57- 1.60)	31 (23.3)	1.73 (0.90- 3.34)	37 (18.8)	0.99 (0.60- 1.65)
13	46 (23.6)	0.96 (0.59- 1.58)	26 (19.6)	1.01 (0.51- 1.99)	55 (27.9)	1.17 (0.72- 1.90)
14	40 (20.5)	0.86 (0.52- 1.43)	32 (24.1)	1.24 (0.64- 2.41)	35 (17.8)	0.76 (0.45- 1.29)
>14	43 (22.1)	1.07 (0.64- 1.78)	29 (21.8)	1.26 (0.64- 2.49)	39 (19.8)	0.97 (0.57- 1.63)
Unknown	6 (3.1)		2 (1.5)		5 (2.5)	
***P* trend**		0.847		0.874		0.506
Cumulative duration of menstrual cycling, accounting for OC use, years [c]						
<23	26 (13.3)	1.00 (referent)	13 (9.8)	1.00 (referent)	33 (16.6)	1.00 (referent)
23- <30	27 (13.9)	0.62 (0.35- 1.09)	30 (22.6)	1.86 (0.93- 3.71)	39 (19.8)	0.99 (0.60- 1.61)
30- <35	37 (19.0)	0.55 (0.31- 0.96)	33 (17.3)	1.18 (0.56- 2.49)	47 (23.9)	1.05 (0.64- 1.74)
≥35	64 (32.8)	0.75 (0.43- 1.28)	31 (23.3)	1.24 (0.58- 2.64)	45 (22.8)	1.15 (0.67- 1.97)
Unknown	41 (21.0)	0.93 (0.53- 1.64)	36 (27.1)	1.81 (0.87 -3.77)	33 (16.8)	0.73 (0.40- 1.33)
***P* trend**		0.863		0.857		0.725
Use of OC						
No	123 (63.1)	1.00 (referent)	64 (48.1)	1.00 (referent)	90 (45.7)	1.00 (referent)
Yes	68 (34.9)	0.84 (0.60- 1.18)	66 (49.6)	1.07 (0.72- 1.59)	102 (51.8)	0.93 (0.67- 1.28)
Unknown	4 (2.1)		3 (2.3)		5 (2.5)	
Duration OC use, years						
No	123 (63.1)	1.00 (referent)	64 (48.1)	1.00 (referent)	90 (45.7)	1.00 (referent)
>0- ≤1	11 (5.6)	0.71 (0.38- 1.33)	4 (3.0)	0.38 (0.14- 1.06)	19 (9.6)	0.85 (0.51- 1.44)
>1- 5	15 (7.7)	0.69 (0.40- 1.21)	17 (12.8)	1.03 (0.58- 1.82)	30 (15.2)	1.08 (0.69- 1.68)
>5- 10	20 (10.3)	1.20 (0.72- 1.99)	24 (18.1)	1.76 (1.05- 2.95)	23 (11.7)	0.93 (0.57- 1.53)
>10	17 (8.7)	0.93 (0.53- 1.61)	9 (6.8)	0.59 (0.28- 1.24)	25 (12.7)	0.92 (0.57- 1.51)
Unknown duration	5 (2.6)		12 (9.0)		5 (2.5)	
Missing use of OC	4 (2.1)		3 (2.3)		5 (2.5)	
***P* trend**		0.359		0.720		0.615
Menopausal status						
Premenopausal	18 (9.5)	1.00 (referent)	9 (6.8)	1.00 (referent)	22 (11.2)	1.00 (referent)
Perimenopausal	19 (10.0)	1.05 (0.46- 2.39)	100 (75.2)	1.48 (0.46- 4.78)	140 (71.1)	3.57 (1.55- 8.24)
Natural postmenopausal	150 (78.9)	0.78 (0.34- 1.78)	18 (13.5)	1.22 (0.39- 3.89)	27 (13.7)	2.31 (1.01- 5.30)
Surgical postmenopausal	8 (1.6)	1.07 (0.38- 3.05)	6 (4.5)	2.06 (0.51- 8.33)	8 (4.1)	3.81 (1.33- 10.94)
Age at natural menopause, years [d]						
≤46	25 (16.7)	1.15 (0.67- 1.93)	19 (19.0)	1.01 (0.55- 1.85)	41 (29.3)	1.23 (0.76- 1.97)
47- 49	26 (17.3)	1.25 (0.75- 2.10)	16 (16.0)	1.14 (0.60- 2.15)	26 (18.6)	0.92 (0.54- 1.55)
50 - 52	36 (24.0)	1.00 (referent)	26 (26.0)	1.00 (referent)	35 (25.0)	1.00 (referent)

≥53	35 (23.3)	1.25 (0.75- 2.10)	22 (22.0)	1.27 (0.71- 2.29)	19 (13.6)	1.12 (0.63- 2.00)
Unknown	28 (18.7)	1.84 (1.07- 3.16)	17 (17.0)	1.07 (0.55- 2.10)	19 (13.6)	1.05 (0.57- 1.93)
***P* trend**		0.532		0.592		0.562
Age at any menopause, years						
≤46	29 (18.4)	1.11 (0.68- 1.81)	24 (22.6)	1.13 (0.64- 2.00)	47 (31.8)	1.28 (0.81- 2.02)
47- 49	26 (16.5)	1.13 (0.68- 1.88)	16 (15.1)	1.05 (0.56- 1.97)	28 (18.9)	0.96 (0.57- 1.60)
50 - 52	39 (24.7)	1.00 (referent)	27 (25.5)	1.00 (referent)	35 (23.7)	1.00 (referent)
≥53	36 (22.8)	1.44 (0.91- 2.29)	22 (20.8)	1.25 (0.70- 2.22)	19 (12.8)	1.13 (0.64- 2.02)
Unknown	28 (17.7)	1.75 (1.02- 2.97)	17 (16.0)	1.05 (0.54- 2.03)	19 (12.8)	1.07 (0.59- 1.96)
***P* trend**		0.464		0.954		0.424
Use of MHT [e]						
No	105 (59.3)	1.00 (referent)	63 (47.4)	1.00 (referent)	77 (39.1)	1.00 (referent)
Yes	52 (29.4)	1.02 (0.71- 1.47)	45 (33.8)	1.21 (0.80- 1.84)	73 (37.1)	1.58 (1.12- 2.23)
Unknown	20 (11.3)	1.14 (0.58- 2.25)	25 (18.8)	0.87 (0.41- 1.85)	47 (23.9)	2.55 (1.34- 4.86)
Duration MHT use, years [e]						
No	105 (59.3)	1.00 (referent)	63 (47.4)	1.00 (referent)	77 (39.1)	1.00 (referent)
>0- ≤1.25	18 (10.2)	1.16 (0.69- 1.95)	10 (7.5)	1.07 (0.54- 2.11)	22 (11.2)	1.73 (1.06- 2.82)
>1.25-4	12 (6.8)	0.87 (0.47- 1.62)	14 (10.5)	1.50 (0.82- 2.76)	21 (10.7)	1.87 (1.12- 3.10)
>4	19 (10.7)	1.24 (0.73- 2.11)	14 (10.5)	1.23 (0.66- 2.30)	22 (11.2)	1.26 (0.75- 2.11)
Unknown duration	3 (1.7)		7 (5.3)		8 (4.1)	
Unknown use of MHT	20 (11.3)		25 (18.8)			
***P* trend**		0.567		0.412		0.421
Type of MHT [e, f]						
Non-users of MHT	88 (63.8)	1.00 (referent)	52 (57.1)	1.00 (referent)	73 (52.5)	1.00 (referent)
Oestrogen alone	7 (5.1)	0.87 (0.40- 1.92)	8 (8.8)	1.41 (0.65- 3.07)	17 (12.2)	2.08 (1.19- 3.62)
Oestrogen + Progestin	22 (15.9)	1.22 (0.72- 2.08)	14 (15.4)	1.21 (0.63- 2.32)	13 (9.4)	0.79 (0.42- 1.48)
Unknown type of MHT	21 (15.2)	1.10 (0.67- 1.80)	17 (18.7)	1.49 (0.84- 2.66)	36 (25.9)	1.68 (1.10- 2.56)
Oophorectomy [g]						
No	141 (82.0)	1.00 (referent)	76 (70.4)	1.00 (referent)	125 (74.4)	1.00 (referent)
Unilateral	9 (5.2)	1.21 (0.61- 2.40)	6 (5.6)	1.03 (0.44- 2.39)	13 (7.7)	1.51 (0.84- 2.70)
Bilateral	8 (4.7)	0.91 (0.44- 1.87)	6 (5.6)	1.21 (0.52- 2.83)	9 (5.4)	1.25 (0.62- 2.52)
Unknown if unilateral or bilateral			1 (0.93)			
Unknown	14 (8.1)	0.07 (0.00- 1.29)	19 (17.6)	1.25 (0.45- 3.48)	21 (12.5)	2.00 (0.79- 5.03)
Hysterectomy [g]						
No	139 (80.8)	1.00 (referent)	76 (70.4)	1.00 (referent)	127 (75.6)	1.00 (referent)
Yes	23 (13.4)	0.83 (0.53- 1.30)	20 (18.5)	1.11 (0.67- 1.84)	32 (19.1)	1.38 (0.92- 2.08)
Unknown	10 (5.8)	0.61 (0.19- 1.95)	12 (11.1)	1.22 (0.42- 3.53)	9 (5.4)	0.89 (0.27- 2.94)
Parity						
No	19 (9.7)	1.00 (referent)	26 (19.6)	1.00 (referent)	27 (13.7)	1.00 (referent)
Yes	170 (87.2)	1.23 (0.76- 1.99)	103 (77.4)	0.61(0.39- 0.95)	164 (83.3)	1.35(0.51- 3.61)
Unknown	6 (3.1)		4 (3.0)		6 (3.1)	
Number of full-term pregnancies [h]						
0 [i]	19 (9.8)	0.72 (0.40- 1.28)	25 (19.7)	1.17 (0.67- 2.06)	27 (12.8)	0.81 (0.48- 1.35)
1	32 (16.6)	1.00 (referent)	26 (20.5)	1.00 (referent)	40 (21.4)	1.00 (referent)
2	83 (43.0)	0.96 (0.63- 1.45)	36 (28.4)	0.57 (.34- 0.96)	72 (38.5)	0.78 (0.52- 1.16)

3	39 (20.2)	0.85 (0.52- 1.37)	25 (19.7)	0.74 (0.42- 1.31)	24 (12.8)	0.47 (0.27- 0.79)
4	9 (4.7)	0.56 (0.26- 1.20)	11 (8.7)	0.93 (0.45- 1.93)	15 (8.0)	1.00 (0.54- 1.85)
≥5	5 (2.6)	0.48 (0.18- 1.28)	0 (0)		6 (3.2)	0.77 (0.32- 1.86)
Unknown parity	6 (3.1)		4 (3.2)		6 (3.2)	
***P*-trend[j]**		0.064		0.208		0.127
Age at first full-term pregnancy, years [j]						
≤20	19 (11.2)	1.00 (referent)	13 (12.6)	1.00 (referent)	36 (22.0)	1.00 (referent)
21- 23	40 (23.5)	0.95 (0.55- 1.65)	32 (31.1)	1.31 (0.68- 2.51)	45 (27.4)	0.91 (0.58- 1.44)
24- 25	34 (20.0)	0.90 (0.51- 1.61)	15 (14.6)	0.77 (0.36- 1.66)	24 (14.6)	0.79 (0.46- 1.35)
26- 30	57 (33.5)	0.93 (0.54- 1.58)	35 (34.0)	1.18 (0.61- 2.29)	47 (28.7)	1.01 (0.64- 1.60)
≥30	20 (11.8)	0.98 (0.51- 1.86)	7 (6.8)	0.73 (0.28- 1.85)	12 (7.3)	0.78 (0.40- 1.54)
Unknown			1 (1.0)			
P-trend		0.906		0.552		0.745
Breastfeeding [j, k]						
No	24 (14.9)	1.00 (referent)	9 (9.9)	1.00 (referent)	24 (15.7)	1.00 (referent)
Yes	133 (82.6)	0.78 (0.50- 1.22)	79 (86.8)	1.17 (0.58- 2.38)	127 (83.0)	0.70 (0.45- 1.11)
Unknown	4 (2.5)		3 (3.3)		2 (1.3)	
Duration of breastfeeding, all pregnancies, months [k, l]						
>0-≤3	49 (36.8)	1.00 (referent)	28 (35.4)	1.00 (referent)	38 (29.9)	1.00 (referent)
>3- 12	49 (36.8)	0.51 (0.34- 0.78)	32 (40.5)	0.60 (0.36- 1.02)	61 (48.0)	1.00 (0.65- 1.53)
>12	34 (25.6)	0.47 (0.29- 0.76)	19 (24.1)	0.78 (0.42- 1.44)	25 (19.7)	1.02 (0.60- 1.76)
Unknown	1 (0.8)					
P-trend		0.015		0.341		0.937
Induced abortions [m]						
Never pregnant	14 (9.0)	0.90 (0.51- 1.59)	17 (19.8)	1.77 (1.01- 3.09)	16 (11.3)	1.05 (0.61- 1.81)
0	114 (73.1)	1.00 (referent)	56 (65.1)	1.00 (referent)	98 (68.0)	1.00 (referent)
1	15 (9.6)	1.29 (0.73- 2.26)	9 (10.5)	1.23 (0.58- 2.86)	21 (14.8)	1.04 (0.63- 1.69)
≥2	12 (7.7)	2.52 (1.33- 4.78)	2 (2.3)	0.65 (0.15- 2.74)	5 (3.5)	0.43 (0.17- 1.08)
Unknown	1 (0.6)		2 (2.3)		2 (1.4)	
***P*-trend**		0.012		0.091		0.175
Spontaneous abortions [n]						
Never pregnant	16 (8.9)	0.84 (0.49- 1.42)	20 (18.0)	1.65 (0.99- 2.77)	19 (11.1)	1.16 (0.68- 1.84)
0	120 (67.0)	1.00 (referent)	67 (60.4)	1.00 (referent)	108 (63.2)	1.00 (referent)
1	35 (19.6)	1.26 (0.86- 1.84)	15 (13.5)	0.91 (0.52- 1.60)	27 (15.8)	1.08 (0.71- 1.67)
≥2	7 (3.9)	0.69 (0.32- 1.49)	6 (5.4)	1.06 (0.46- 2.46)	14 (8.2)	1.52 (0.86- 2.68)
Unknown	1 (0.6)		3 (2.7)		3 (1.8)	
***P*-trend**		0.679		0.185		0.375
Infertility problems [o]						
No	122 (89.7)	1.00 (referent)	57 (79.2)	1.00 (referent)	75 (77.3)	1.00 (referent)
Yes	4 (2.9)	0.93 (0.34- 2.55)	7 (9.7)	3.12(1.38- 7.04)	5 (5.2)	1.32(0.50- 3.49)
Unknown	10 (7.4)		8 (11.1)	2.34(0.95- 5.74)	17 (17.5)	0.44(0.12- 1.55)

UC: urothelial carcinoma // OC: oral contraceptive // MHT: menopause hormone therapy

All *P* value for the interaction were >0.05, with the exception of the induced abortions were *P* for interaction = 0.028

[a] Cox proportional hazards model stratified by center and age at recruitment and adjusted by fruits and vegetables intake.

[b] Cox proportional hazards model stratified by center and age at recruitment and adjusted by smoking intensity (number of cigarettes per day in current-smokers and time since quitting smoking in former-smokers), fruits and vegetables intake.

[c] Cox proportional hazards model stratified by center and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake, OC use, and full-term pregnancies

[d] Women who had surgical menopause were excluded

[e] In peri- and postmenopausal (natural or surgical).

[f] Available in France, Italy, Spain, United kingdom, The Netherlands, Germany, Denmark, and Norway.

[g] Available in all centers except Malmö.

[h] Available in all centers except Bilthoven.

[i] Including nulliparous women and women without full-term pregnancies.

[j] In parous women.

[k] Available in all centers except Bilthoven and Umeå.

[l] In parous women who has ever breastfed.

[m] Available in all centers except Bilthoven, Malmö, Umeå, and Norway.

[n] Available in all centers except Bilthoven, Umeå, and Norway.

[o] Available in France, Italy, Spain, United Kingdom, Utrecht, Greece, and Germany.

Table 17: Supplemental table 4: Mutually adjusted models for menopause status, MHT, and parity, and UC by smoking status

	Never		Former		Current	
	Cases (%) n =195	HR (95%CI)[a]	Cases (%) n =133	HR (95%CI)[b]	Cases (%) n =197	HR (95%CI)[b]
Menopausal status & use of MHT						
Premenopausal	18 (9.23)	1.23 (0.52- 2.43)	9 (6.8)	0.83 (0.27- 2.54)	22 (11.2)	0.50 (0.22- 1.11)
Peri-/Postmenopausal & non-users of MHT	105 (53.9)	1.00 (referent)	63 (47.4)	1.00 (referent)	77 (39.1)	1.00 (referent)
Peri-/Postmenopausal & users of MHT	52 (26.7)	1.02 (0.71- 1.47)	45 (33.8)	1.20 (0.79- 1.83)	73 (37.1)	1.56 (1.10- 2.21)
Peri-/Postmenopausal & unknown MHT-use	20 (10.26)	1.12 (0.53- 2.39)	16 (12.0)	0.89 (0.40- 2.00)	25 (12.7)	2.31 (1.16- 4.62)
Number of full-term pregnancies [c]						
0 [d]	19 (9.7)	0.72 (0.40- 1.29)	26 (19.6)	1.17 (0.67- 2.06)	27 (13.7)	0.83 (0.49- 1.39)
1	32 (16.4)	1.00 (referent)	26 (19.6)	1.00 (referent)	40 (20.3)	1.00 (referent)
2	83 (42.6)	0.95 (0.63- 1.45)	36 (27.1)	0.57 (0.34- 0.96)	72 (36.6)	0.78 (0.49- 1.39)
3	39 (20.0)	0.85 (0.52- 1.37)	25 (18.8)	0.74 (0.42- 1.30)	24 (12.2)	0.48 (0.28- 0.81)
4	9 (4.6)	0.57 (0.27- 1.21)	11 (8.3)	0.94 (0.45- 1.95)	15 (7.6)	1.01 (0.54- 1.88)
≥5	5 (2.6)	0.49 (0.18- 1.29)			6 (3.1)	0.80 (0.33- 1.95)
Unknown	8 (4.1)		9 (6.8)		13 (6.6)	
***P*-trend [e]**		0.069		0.209		0.149

UC: urothelial carcinoma // MHT: menopause hormone therapy
All *P* value for the interaction were >0.10, with the exception of the induced abortions were *P* for interaction = 0.028
[a] Cox proportional hazards model stratified by center and age at recruitment and adjusted by fruits and vegetables intake.
[b] Cox proportional hazards model stratified by center and age at recruitment and adjusted by smoking intensity (number of cigarettes per day in current-smokers and time since quitting smoking in former-smokers) , fruits and vegetables intake.
[c] Available in all centers except Bilthoven.
[d] Including nulliparous women and women without full-term pregnancies.
[e] In parous women.

10.5. Curriculum Vitae and list of main publications

10.5.1. Curriculum Vitae

The author of this doctoral thesis, Leila Luján, was born in Barcelona in 1982. In 2003, she obtained a degree in Statistic from Polytechnic University of Catalonia and in 2006 she obtained MSc in Statistics, Statistical Sciences, and Techniques from the same University.

From 2007 to 2008, she worked at the Sant Joan de Déu Fundation, her main responsibilities were development of statistical analyses in the field of mental health and environmental factors. In 2008, she started to work at the Catalan Institute of Oncology where she mainly works on the European Prospective Investigation into Cancer and Nutrition (EPIC) assessing the impact of environmental factors on cancer. In 2014, she started to work on the International Pancreatic Cancer case-control (PanC4) consortium analyzing the relation between menstrual and reproductive factors, exogenous hormone use, and the risk of pancreatic cancer. Furthermore, she collaborates in the epigenetic analysis for both the EPIC and PanC4 projects. In 2014, she started to work as an associate professor at University of Barcelona teaching a course on Introduction to Scientific Methodology for second-year nursing students.

10.5.2. List of main publications

The following list of publications includes the papers in which Leila Luján has contribute in a significant way. Articles presented in the present doctoral thesis are not included in the list.

- Obón-Santacana M, **Luján-Barroso L**, Freisling H, *et al.* Consumption of nuts and sedes and pancreatic ductal adenocarcinomar risk in the European Prospective Invesetigation into Cancer and Nutrition. Int J Cancer. 2020 Jan 1;146(1):76-84. doi: 10.1002/ijc.32415. Epub 2019 Jun 6.
- Jakszyn P, Fonseca-Nunes A, **Lujan-Barroso L**, *et al.* Hepcidin levels and gastric cáncer risk in the EPIC-EurGast study. Int J Cancer. 2017 Sep 1;141(5):945-951.
- Duell EJ, **Lujan-Barroso L**, Sala N, *et al.* Plasma microRNAs as biomarkers of pancreatic cáncer risk in a prospective cohort study. Int J Cancer. 2017 Sep 1;141(5):905-915.

- Buckland G, Pastor A, **Lujan-Barroso L**, *et al.* Determination of aleanolic acid in human plasma and its association with olive oil intake in healthy Spanish adults within the EPIC Spain cohor study. Mol Nutr Food Res. 2017 Aug;61(8).
- Obón-Santacana M, **Lujan-Barroso L**, Freisling H, *et al.* Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort. Eur J Nutr. 2017 Apr;56(3):1157-1168.
- Obón-Santacana M, **Lujan-Barroso L**, Travis RC, *et al.* Acrylamide and Glycidaminde Hemoglobin Adducts and Epithelial Ovarian Cancer: A Nested Case-Control Study in Nonsmoking Postmenopausal Women from the EPIC cohort. Cancer Epidemiol Biomarkers Prev. 2016 Jan;25(1):127-34.
- Zamora-Ros R, **Luján-Barroso L**, Bueno-de-Mesquita HB, *et al.* Tea and coffee consumption and risk of esophageal cáncer: the European prospective investigation into cáncer and nutrition study. Int J Cancer. 2014 Sep 15;135(6):1470-9.
- **Lujan-Barroso L**, González CA, Slimani N, *et al.* Dietary intake of acrylamide and esophageal cáncer risk in the European Prospective Investigation into Cancer and Nutrition cohort. Cancer Causes Control. 2014 May;25(5):639-46.
- Obón-Santacana M, Slimani N, **Lujan-Barroso L**, *et al.* Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Ann Oncol. 2013 Oct;24(10):2645-51.
- Duell EJ, **Lujan-Barroso L**, Llivina C, *et al.* Vitamin C transporter gene (SLC23A1 and SLC23A2) polymorphisms, plasma vitamin C levels, and gastric cancer risk in the EPIC cohort. Genes Nutr. 2013 Nov;8(6):549-60.
- Jakszyn P, **Luján-Barroso L**, Agudo A, *et al.* Meat and heme iron intake and esophageal adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition study
- Int J Cancer. 2013 Dec 1;133(11):2744-50.
- Zamora-Ros R, Agudo A, **Luján-Barroso L**, *et al.* Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr. 2012 Dec;96(6):1398-408.

- Duell EJ, Travier N, **Lujan-Barroso L**, *et al*. Menstrual and reproductive factors in women, genetic variation in CYP17A1, and pancreatic cancer risk in the European prospective investigation into cancer and nutrition (EPIC) cohort. Int J Cancer. 2013 May 1;132(9):2164-75.
- Zamora-Ros R, Knaze V, **Luján-Barroso L**, *et al.* Differences in dietary intakes, food sources and determinants of total flavonoids between Mediterranean and non-Mediterranean countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 2013 Apr 28;109(8):1498-507.
- Zamora-Ros R, Knaze V, **Luján-Barroso L**, *et al.* Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. Eur J Clin Nutr. 2012 Aug;66(8):932-41.
- Gonzalez CA, **Lujan-Barroso L**, Bueno-de-Mesquita HB, *et al.* Fruit and vegetable intake and the risk of gastric adenocarcinoma: a reanalysis of the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study after a longer follow-up. Int J Cancer. 2012 Dec 15;131(12):2910-9.
- Jakszyn PG, Allen NE, **Lujan-Barroso** L, *et al.* Nitrosamines and heme iron and risk of prostate cancer in the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev. 2012 Mar;21(3):547-51.
- Knaze V, Zamora-Ros R, **Luján-Barroso L**, *et al.* Intake estimation of total and individual flavan-3-ols, proanthocyanidins and theaflavins, their food sources and determinants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 2012 Sep 28;108(6):1095-108.
- Serafini M, Jakszyn P, **Luján-Barroso L**, *et al.* Dietary total antioxidant capacity and gastric cancer risk in the European prospective investigation into cancer and nutrition study. Int J Cancer. 2012 Aug 15;131(4):E544-54.
- Duell EJ, Travier N, **Lujan-Barroso L**, *et al.* Alcohol consumption and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Am J Clin Nutr. 2011 Nov;94(5):1266-75.
- Jakszyn P, Agudo A, **Lujan-Barroso L**, *et al.* Dietary intake of heme iron and risk of gastric cancer in the European prospective investigation into cancer and nutrition study. Int J Cancer. 2012 Jun 1;130(11):2654-63.

- Zamora-Ros R, Knaze V, **Luján-Barroso L**, *et al.* Estimated dietary intakes of flavonols, flavanones and flavones in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24 hour dietary recall cohort. Br J Nutr. 2011 Dec;106(12):1915-25.
- Zamora-Ros R, Knaze V, **Luján-Barroso L**, *et al.* Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 2011 Oct;106(7):1090-9.
- Jakszyn P, González CA, **Luján-Barroso L**, *et al.* Red meat, dietary nitrosamines, and heme iron and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Epidemiol Biomarkers Prev. 2011 Mar;20(3):555-9.
- González CA, Travier N, **Luján-Barroso L**, *et al.* Dietary factors and in situ and invasive cervical cancer risk in the European prospective investigation into cancer and nutrition study. Int J Cancer. 2011 Jul 15;129(2):449-59.
- Duell EJ, Travier N, **Lujan-Barroso L**, *et al.* Menstrual and reproductive factors, exogenous hormone use, and gastric cancer risk in a cohort of women from the European Prospective Investigation Into Cancer and Nutrition. Am J Epidemiol. 2010 Dec 15;172(12):1384-93.
- Buckland G, Agudo A, **Luján L**, *et al.* Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. Am J Clin Nutr. 2010 Feb;91(2):381-90.