UiT The Arctic University of Norway

Faculty of health sciences / Department of community medicine

Intake of Fat and the Risk of Epithelial Ovarian Cancer in the Norwegian Women and Cancer Study

Frida Stormo Magnussen

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Abbreviations

BMI	Body mass index
CI	Confidence interval
DAG	Directed acyclic graph
EOC	Epithelial ovarian cancer
FFQ	Food frequency questionnaire
g/d	Grams per day
HDL	High density lipoprotein
HR	Hazard ratio
IQR	Interquartile range
kJ	kilojoule
LDL	Low density lipoprotein
LNYM	Lifetime number of years menstruation
mg/d	Milligrams per day
NOWAC	Norwegian Women and Cancer
OC	Ovarian Cancer
Q	Quartile
REK	Regionale komiteer for medisinsk og helsefaglig forskningsetikk
SD	Standard deviation
WCRF	World Cancer Research Fund
WHO	World Health Organisation

Abstract

Background: Worldwide, ovarian cancer is the 8th most common cancer type for women. According to The World Cancer Research Fund, the research on the associations between intake of fat and risk of ovarian cancer is limited, and more research is needed.

Aim: To study the possible associations between the intake of total fat, trans fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids, cholesterol and the risk of developing epithelial ovarian cancer (EOC) among the participants in the Norwegian Woman and Cancer (NOWAC) study.

Material and methods: This thesis included 90 792 Norwegian women from the NOWAC study who by random selection were recruited through the National Population Register from 1996 to 2004. The participants were 41 to 76 years at baseline. The dietary data was collected through self-reported food frequency questionnaires, and the intake of fat was calculated from these. Intake of fat was divided into quartiles and the lowest intake group was used as the reference group for all analyses. Cox proportional hazards models were used to assess hazard ratios (HR) and 95% confidence intervals (CI) to study the associations between intake of total fat, different fatty acids, cholesterol and risk of EOC.

Results: 596 cases of EOC were identified during follow up. All p-values were >0.05 and no associations were found when adjusting for age, education, or family history of breast cancer in the analyses.

Conclusion: Overall, no associations between the intake of total fat, trans fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids and cholesterol and risk of developing EOC among the participants in the NOWAC study was observed. However, there are inconclusive results reported from other studies, and more research is needed on this topic.

Sammendrag

Bakgrunn: Eggstokkreft er den åttende mest vanlige krefttypen som rammer kvinner på verdensbasis. Ifølge World Cancer Research Fund er evidensen for inntaket av fett og risiko for eggstokkreft begrenset, og mer forskning på dette feltet er nødvendig.
Formål: Å undersøke assosiasjonen mellom inntaket av totalt fett, transfett, flerumettet fett, enumettet fett, mettet fett, kolesterol og risiko for eggstokkreft blant middelaldrende kvinner i Kvinner og Kreft-studien.
Material og metoder: Selvrapportert kostholdsinformasjon ble innhentet gjennom matvarefrekvensskjema fra 90 792 norske kvinner fra Kvinner og Kreft-studien. Deltagerne ble tilfeldig valgt ut gjennom folkeregisteret fra 1996-2004 og var mellom 41-76 år ved inklusjon. Cox proporsjonal hasard regresjonsanalyse ble brukt til å beregne hasard ratio (HR) og 95% konfidensintervaller (CI) for å estimere sammenhengen mellom inntaket av fett og risiko for eggstokkreft. Fettinntaket ble beregnet basert på matvarefrekvensskjema, og kategorisert i kvartiler for analysene. Gruppen med lavest inntak ble brukt som referanse-gruppe for alle analyser.
Resultater: 596 tilfeller av eggstokkreft blant deltagerne ble rapportert. Alle p-verdier var >0.05 og resultatene viste ingen assosiasjon ved justering av alder, utdanning og familiehistorie for brystkreft i analysene.
Konklusjon: Det ble ikke funnet en signifikant sammenheng mellom inntaket av totalt fett, transfett, flerumettet fett, enumettet fett, mettet fett, kolesterol og risiko for epitelial eggstokkreft. Derimot viser andre studier varierende resultater som understreker behovet for mer forskning på feltet.

Foreword

First of all, I have to thank my extraordinary supervisor, Marie Hauan. If I didn’t have you through this, to cheer me on, give me guidance and feedback beyond what I could ever expect, this would not have been possible. Your patience is simply admirable!

Further I must thank my fellow students, and especially Caroline and Vanja. Writing this thesis would not have been the same without you. I also want to express my gratitude to my dear friends, Tuva and Sara. Your help was extremely appreciated and thank you for believing in me when I didn’t believe in myself.

1. Introduction

1.1 Background

In 2022, 506 women were diagnosed with ovarian cancer (OC) in Norway, and the 5 years relative survival rate from 2018 to 2022 was 51.2% (1). The median age for developing OC in Norway is 68 years, and 40% of Norwegian women diagnosed in 2022 was younger than 40 years old. 303 women died of OC in Norway during 2022, and 207 252 women died worldwide of the same cause (1, 2). The accumulated risk of developing OC until the age of 80 years old is 1.5%, compared to breast cancer which was 10.7% (2018-2022) (1, 3). Worldwide, OC is the 8th most common cancer type for women, and in addition OC was ranked nr 14 regarding mortality (2, 4).

The reproductive system consists of two ovaries, vulva, vagina, uterus, and fallopian tubes as shown in figure 1. The ovaries produce, stores and releases eggs that can result in a pregnancy. Additionally, the ovaries produce sex hormones, estrogen and progesterone, until a woman reaches menopause (5).

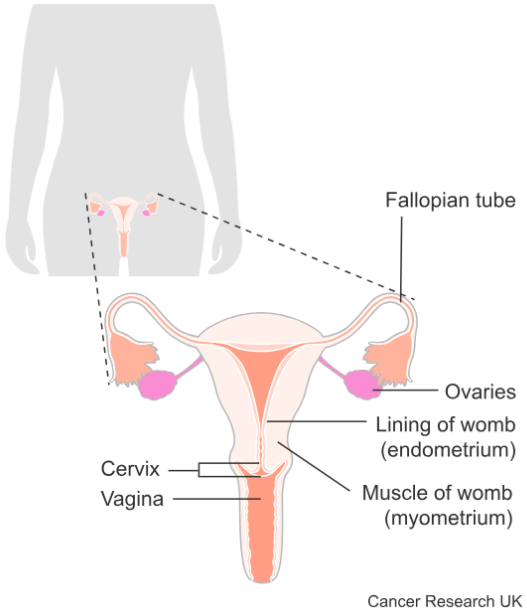

Figure 1 The reproductive system (5).

1.2 Epithelial Ovarian Cancer

There are different types of ovarian cancer, but the most common type is epithelial ovarian cancer (EOC), which covers about 90% of the cases (6). EOC develops when epithelial cells of the ovary are damaged, resulting in mutations of genes. The mutation can cause the

epithelial cells to proliferate uncontrollably, through activation of the pathways involved in cell cycle regulation and accumulate (7, 8). Epithelial cells have rapid turnover, which increases the risk of developing cancer as women get older (7). Without early detection, these cancer cells can progressively invade nearby tissues and can potentially metastasize to other parts of the body (9). EOC can be put into a stage and grade system, based on how far the cancer has grown and developed. Stage 1 means the cancer is only in the ovaries, meanwhile in stage 4 the cancer has spread to not only close organs, but also further (8). There are little to none distinctly symptoms of ovarian cancer, therefore EOC is often diagnosed at a late stage with poor prognosis. Symptoms can be abdominal bloating, back pain, constipations or diarrhoea, unexplained weight loss and tiredness; all which could be caused by other conditions. If detected at stage 1, the 5-years-survival-rate is 99.9%, meanwhile the survival rate drop to 37.9% if detected at stage 4 (1).

The risk factors for EOC is not fully researched, but there is evidence that not bearing children, early menarche, late menopause, use of hormone replacement treatment, use of tobacco and having a family history of EOC increase the risk (2). In addition, there is also compelling evidence that being tall and/or obese increases the risk. Meanwhile, sterilisation decreases the risk, and breastfeeding and use of oral contraceptives might decrease the risk of EOC (2).

1.3 Dietary fats

Fat and fatty acids are the most nutrient-dense macronutrient with 9 kcal per gram (10). Four main types of lipids are triglycerides, phospholipids, cholesterol, and free fatty acids. Our diets usually consist of fats in form of triglycerides, which consists of glycerol connected with three fatty acids (11). Triglycerides differs in length of carbon chain, degree of saturation, and the placement of double bonds. All fatty acids contains of one carboxylic acid (COOH), following a number of carbon atoms and with a methyl group (CH_3) at the other end (11). Fat can also be divided into saturated fat, monounsaturated fat, polyunsaturated fat and trans-fat. Fat has many functions and are involved in many processes in the human body, such as signalling, energy source and storage, cell structure, metabolism, hormones and protein modification (11). The total intake of fat should be 25-40 E%, according to The Norwegian Directorate of Health (12). Energy percentage (E%), is the proportions of the total energy content of the diet that come from the different macronutrients. An intake of total fat lower than 20-25 E% is unfortunate considering the need for essential fatty acids, fat-soluble

vitamins and it is connected to reduced HDL cholesterol, increased triglycerides in the blood and decreased glucose tolerance in some individuals (13). However, high intakes of fat are also related to serious health problems (13).

Saturated fat is characterized by no double bonds, meaning that all the carbon atoms are fully saturated with hydrogen atoms. Saturated fat is known to increase the levels of LDL and HDL cholesterol in the blood (14). The Norwegian Directorate of Health recommend the intake of saturated fat to be below 10 E% (12). The recommendation for saturated fatty acids is also defended by contributing to reduce the intake of trans fatty acids. In 2021 and 2022, the Norwegian population had a saturated fat intake of 14 E% according to the report from Trends in the Norwegian Diet 2022 (15). The biggest sources of saturated fat in the Norwegian diet is butter, margarine, oil, cheese, meat and meat products (15).

Unsaturated fatty acids have double bonds between the carbon atoms, which discourage the tight packing of atoms. This makes it a less stabile molecule, in addition to lowering of the melting point, usually making them more fluid than other types of fat. Unsaturated fatty acids are especially important in absorption of and utilization of the fat-soluble vitamins: A, D, E and K. The Norwegian Directorate of Health recommends an intake of polyunsaturated fat to cover 5-10 E% and 10-20 E% for monounsaturated fat. Trends in the Norwegian Diet 2022 reports an intake of monounsaturated fat and polyunsaturated fat of 14 E% and 6 E% in 2021, which is within the recommendations. Sources to unsaturated fat in the Norwegian diet are fatty fish, oils (olive, rapeseed) and nuts. Unsaturated fat is found to be beneficial for blood cholesterol and lowering the risk of coronary heart diseases (16).

Trans fatty acids are also unsaturated, but are, contrary to unsaturated fat, linked to negative health consequences. Trans fatty acids have trans double bonds instead of cis double bonds, making the fatty acids straight instead of bent, allowing them to be packed tight and straighter, like saturated fat. The recommendations for trans-fat is to be as low as possible, but the intake in Norway was 0.6 E% in 2021 (15). For Norwegian women, the biggest source of trans-fat is processed meat and dairy products (15).

Cholesterol is a type of lipid which can be synthesized in the body and be consumed through the diet. The human body needs cholesterol for hormones, bile acids, transport of fatty acids in the blood and being important components in the cell membranes to keep them fluid (17). The chemical structure of cholesterol is different from the other type of lipids, as cholesterol

exists of a ring system which is non-polar, but also with a hydroxyl group which is polar. This gives cholesterol mixed properties, however when cholesterol is esterified to a long-chain fatty acid, the whole molecule becomes very non-polar (11). Because of its non-polar properties, cholesteryl esters are important components in lipoproteins, which transports lipids through the bloodstream (11). High-density lipoprotein (HDL) is in the everyday language called the ‘’good ’’ cholesterol, because it transports the cholesterol to the liver, which expels it from the body (17). Low-density lipoprotein (LDL) is considered the ‘’bad’’ cholesterol, because it contributes to the buildup of plaque in the arteries causing atherosclerosis (17). A high cholesterol level in the blood can therefore lead to serious coronary artery disease. Cholesterol is common found in foods with a high level of saturated fatty acids, for example meat and full-fat dairy products (17). There are other factors that also effects the levels of cholesterol in the blood such as physical activity, smoking, age, BMI and heredity (17).

1.4 Mechanism of EOC

There are several suggested mechanisms for how total fat intake can affect the risk of EOC. A high consumption of fat can lead to several negative health outcomes such as obesity, insulin resistance, hyperinsulinemia and cause high levels of growth factor-1 receptor. All of these can induce androgen steroidogenesis and result in EOC (18). There is also evidence regarding stimulation of the secretion of oestrogen from the ovaries, when having a high intake of total fat, which further can lead to EOC (19). Trans fatty acids appear to trigger inflammation in the human body and have a negative impact on endothelial function. Also, abundant evidence suggests that chronic inflammation is linked to the process of carcinogenesis and therefore increase the EOC risk (20, 21). Monounsaturated fatty acids are known to decrease the LDL cholesterol and increase the HDL cholesterol in the blood, and in this way also effects the risk of EOC through the risks of having a high cholesterol levels (19). The mechanism behind the suggested theory that cholesterol may contribute to increased risk of EOC is the link between cholesterol being a precursor to the synthesis of steroid hormones, including oestrogen (22).

1.5 Existing research

There is inconsistent and inconclusive research regarding if the intake of fatty acids is affecting the risk of developing EOC. According to the 2018 Ovarian Cancer report from World Cancer Research Fund (WCRF) the intake of total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, trans fatty acids and cholesterol is in

the category of being ‘’limited evidence’’ (23). Further, several meta-analyses and reviews have found either weak associations or not significant results on this matter. Qui et al (24) found a positive associations between the intake of total fat, saturated fatty acids, trans fatty acids and risk of EOC. The same meta-analysis did not find an association for monounsaturated fatty acids and polyunsaturated fatty acids. However, Hou et al (25) only found non-significant results in their meta-analysis, but the results showed there could be a negative association for monounsaturated fatty acids and polyunsaturated fatty acids and positive association for the intake of total fat, saturated fatty acids and trans fatty acids. Merritt et al (26) found a significant higher risk of EOC with a high intake of trans fatty acids and significant lower risk when consuming polyunsaturated fatty acids. However, Merritt et al found no association for the intake total fat, saturated fatty acids, monounsaturated fatty acids or cholesterol. Overall, more research is needed to establish the relationship between intake of fat and the risk of EOC.

2. Aim

The aim for this thesis was to study the possible association between the intake of fat and risk of EOC among the participants in the NOWAC study. More specifically, the objectives were to assess the association between the intake of total fat, trans fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids, cholesterol and the risk of developing EOC.

3. Methods

3.1 The NOWAC study

The Norwegian Women and Cancer (NOWAC) study started researching the relationship between lifestyle factors and the risk of developing cancer in 1991. NOWAC is a population-based prospective cohort study based on questionnaires and blood samples from Norwegian women between 30 and 70 years old at study enrollment. The total cohort is illustrated in Appendix 1. The participants were randomly selected from Norway’s National Population Register, where all residents, and former residents of Norway are registered, and given an identification number (27). The NOWAC study is also linked to the Cancer Registry of Norway and the Norwegian Cause of Death Registry (28). 172 472 women have been included in the study, and they reported information on anthropometry, reproductive history, use of medications, self-reported diseases, socioeconomic status, and lifestyle factors such as

diet, physical activity and use of tobacco. All participants have answered at least one follow-up questionnaire, and a maximum of four.

3.1.1 Dietary data collection

Information on diet was reported using self-reported food frequency questionnaire (FFQs) (29). The questionnaires used in NOWAC have differed regarding questions included, and it was not until 1996 that the FFQ was included as part of the questionnaire. Consequently, not all participants included in the NOWAC study received a FFQ at baseline. The participants that did not receive the FFQ at baseline were given a FFQ at follow-up. The baseline FFQ was sent out in 1996, 1997, 2003 and 2004. The follow-up FFQ was send out in 1998, 1999 and 2002. In the cases where participants did not receive a FFQ at baseline, their FFQ from the follow-up questionnaire was used. Therefore, baseline for this study is 1996 to 2004, and the women were 41-76 years old at enrolment.

The FFQ contained questions about the participants' diet the past year, and they were asked to answer questions regarding their everyday, normal diets. Depending on the food type, the questions were inquired about both quantity and/or frequency. For some food types, such as fish and ice cream, the FFQ contained questions about season variability, because the accessibility can vary, or certain foods are usually more commonly consumed during a specific season. The FFQs were then used to calculate the participants intake of each nutrient. Specifically, standard portion sizes and standard weights were estimated using ''Mål, vekt og porsjonsstørrelser for matvarer'' by The Norwegian Food Safety Authority, University of Oslo and The directorate of Health (30). To determine the nutritional content in each food item ''Matvaretabellen'' (31) was used, which is also developed by The Norwegian Food Safety Authority. Dietary questions which were not filled out was counted as null intake, and if quantity measures were missing, a small standard portion was used in the estimate. Fat used when cooking was not taken into account, hence the report already had calculated this.

3.2 Exposures

The exposures for this thesis are intake of total fat, saturated fat, monounsaturated fat, polyunsaturated fat, trans fatty acids and cholesterol. Total fat, saturated fat, monounsaturated fat, polyunsaturated fat and trans fatty acids were measured in grams per day (g/day), while cholesterol was measured in milligrams per day (mg/d). These units have previously been calculated from the FFQ as described in 3.1.1. The participants were divided into quartiles based on the distribution of total fat intake.

3.3 Inclusion and exclusion

All women who responded to at least one FFQ were included in the analyses. There were 101 316 participants in the raw dataset and 90 729 after exclusions. Specifically, participants were excluded if: they had been diagnosed with any cancer prior to baseline, or died or immigrated before the questionnaire was registered (n =3322), had an unrealistic (above 15 000 kJ or under 2500 kJ) energy intake (n = 1175) or missing data on education (n=6027).

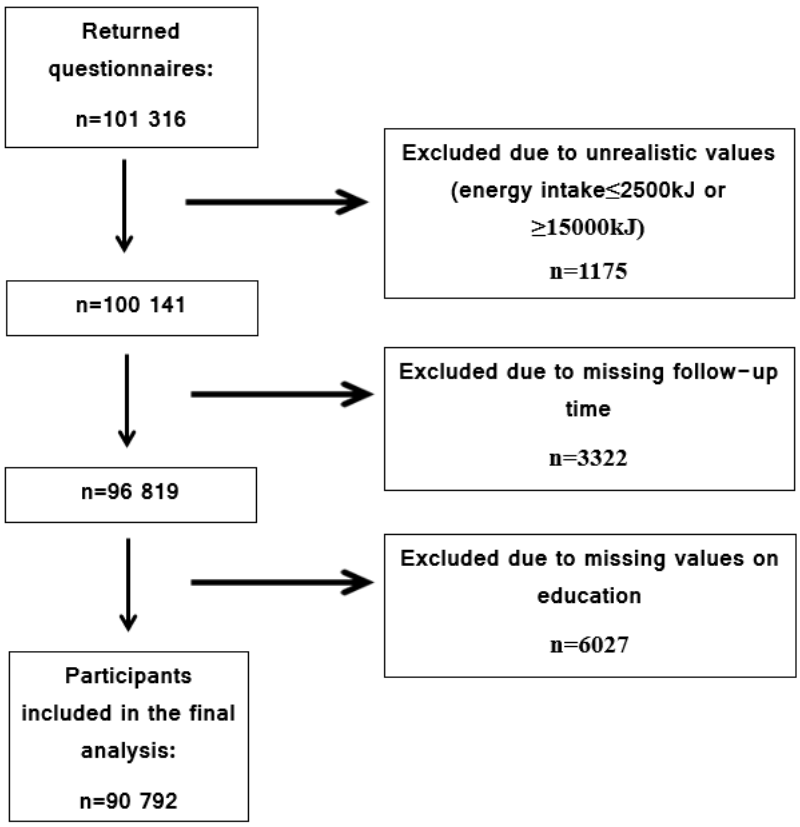

Figure 2 Flowchart of participants included and excluded in the thesis.

3.4 Outcome

Primary invasive epithelial ovarian cancer was the outcome of this study, and defined as ICD-10 code C56 and C569 (32). More specifically, only participants with morphology codes 8010/3, 8020/3, 8041/3, 8070/3, 8140/3, 8246/3, 8255/3, 8260/3, 8310/3, 8380/3, 8440/3, 8441/3, 8450/3, 8460/3, 8461/3, 8470/3, 8480/3, 8560/3, 8570/3, 8574/3, 8951/3, 8980/3, 9000/3, 9110/3 were defined as cases (33). All the morphology codes are EOC. 80-95% of all ovarian cancers are in the epithelial tissue, and EOC is therefore the defined outcome (34). Meanwhile follow-up was continued until the participants emigrated, died, got diagnosed with cancer or 31st of December 2020, whatever occurred first.

3.5 Confounding factors

A directed acyclic graph (DAG) was used to illustrate the relationship between fat intake and the risk of developing EOC, in addition to address which other factors affects the association. Thus, this is how the confounding variables were identified. In addition to the exposure and the outcome, known risk factors of EOC were included in the DAG, illustrated in Figure 3. According to the report from WCRF (23) there is strong evidence that being tall and obese increases the risk of ovarian cancer, meanwhile, breastfeeding might decrease the risk (2). Other contributing factors related to developing ovarian cancer according to the WCRF are oral contraceptives, hormone replacement treatment, family history of ovarian- and breast cancer, and use of tobacco. Additionally, the higher the number of menstrual cycles, the higher risk of ovarian cancer. Therefore, late menarche, early menopause and being pregnant is protective factors (2). Other known factors for developing cancer in general is low physical activity, older age, and alcohol consumption (2). Energy intake can affect several of the factors in the DAG, so total energy intake is also counted for. The confounding variables identified were education and family history of breast cancer.

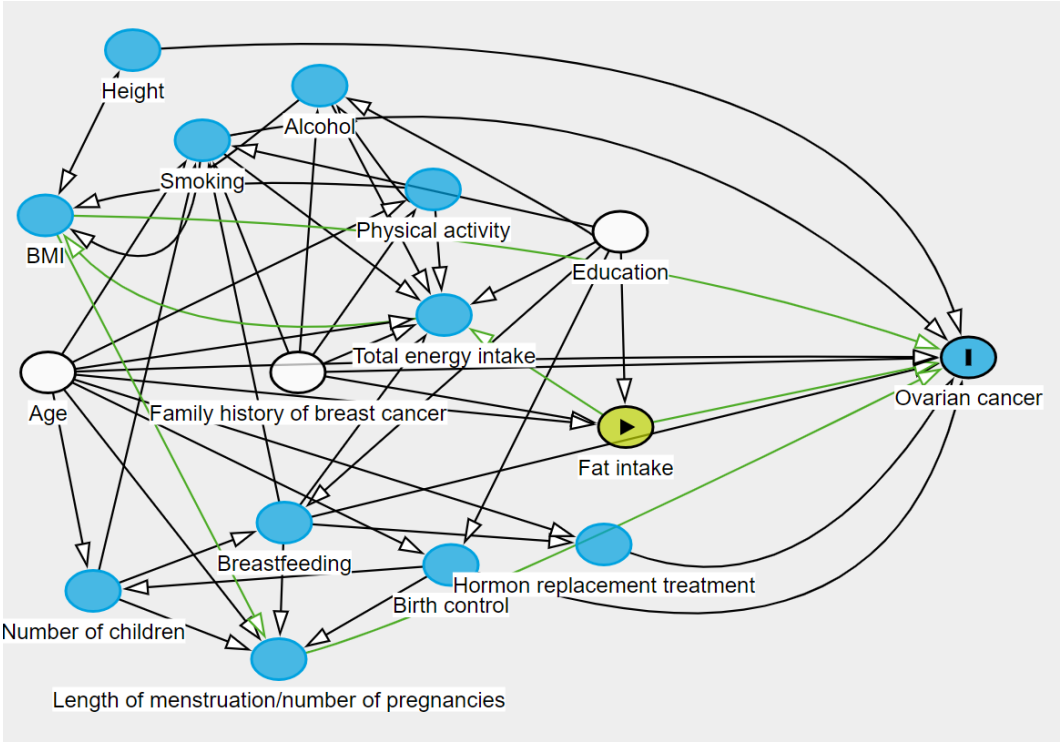

Figure 3 DAG illustrating factors affecting the relationship between intake of fat and EOC.
Green line: causal path
Grey circles: unobserved variables
White circles: adjusted variables
Blue circles: precursor variables of outcome variable
Green circle: exposure variable
Blue circle with I: outcome variable
The figure is created from http://www.dagitty.net/

3.6 Covariates

In addition to the FFQ, which collected dietary data, the participants also responded to a questionnaire to collect information such as age and education. All these types of variables are therefore self-reported data or calculated based on self-reported data such as body mass index (BMI). BMI was divided into four groups based on The World Health Organisation (WHO) classification. Underweight being below 18.5 kg/m^2, normal weight 18.5-24.9 kg/m^2, overweight 25.0-29.0 kg/m^2 and obese over 29.0 kg/m^2 (35). Smoking was divided into three groups: never, former and current smoker. Education was divided into three categories based on number of years at school: under 10 years, 10-12 years and more than 12 years. Physical activity was scored on a scale from 1-10 and divided into three groups: low 1-3, moderate 4-7 and high 8-10. Lifetime number of years of menstruation (LNYM), is a summarized variable that takes into consideration the first menstrual cycle of a women, her menopause, number of pregnancies among other exposures that effects a woman's cumulative exposure to endogenous oestrogen such as breastfeeding and use of oral contraceptives (36). LNYM is a continuous variable in this thesis.

3.7 Validation

The external validity for the NOWAC study has already been assessed. In an article written by Lund et al (37), the results from the analysis showed no selection bias that could lead to serious invalidation. Also, no significant differences were observed when comparing the distribution of smoking, weight, oral contraceptives, parity and education to 5000 randomly drawn women from five different series(C4-C8) with different response-rate (37). As part of the validation, the fertility register and the register of education was used to achieve estimates which were comparable for the non-responders and responders (37). Hjartåker et al (38) compared the FFQ used in the NOWAC study with repeated 24-hour dietary recalls. The results stated that the FFQs were underestimating energy intake and alcohol compared to the 24-hour recalls. For total fat intake, the FFQ showed a median of 59.5 g/day, 95% CI: 48.6-74.2, meanwhile the 24-hour recall found a median of 71.9 g/day, 95% CI: 59.0-88.9 (38). However, the study concluded that foods that were eaten regularly and the intake of macronutrients was accurately reported (38).

3.8 Statistical analyses

To determine the risk of EOC, the statistical program Stata version 18.0 was used (39). Descriptive statistics of age at baseline and total energy intake were presented as mean values with standard deviations. LNYM was reported with median and interquartile range (IQR). Regarding BMI, education, smoking, physical activity and all types of fat, the percentage of the distribution of the participants in each quartile were reported. Cox proportional hazards models were used to assess hazard ratios (HRs) and 95% confidence intervals (CI) for the association between the intake of different types of fat and the risk of EOC. Specifically, the analyses were conducted for total fat, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, trans fatty acids and cholesterol intake. Age was used as the time metric. The analyses were conducted in two models, in addition to a sensitivity analysis. Model 1 adjusted for age and model 2 additionally adjusted for education. The sensitivity analyses adjusted for the same factors as model 2 including family history of breast cancer. Due to missing values, the family history of breast cancer was chosen for the sensitivity analyses. If executed correctly, a sensitivity analysis can increase transparency in research and strengthen the results (40). The level of significance was set as < 0.05. Schoenfeld residuals were visually inspected to see if the assumptions of proportional hazards were fulfilled.

3.9 Ethical considerations

The NOWAC study have already been approved by Regional Committees for Medical and Health Research Ethics (REK) and the work in this thesis is also covered by that approval. The randomly selected women received a letter containing information about the NOWAC study, the right to withdraw consent, the regional ethical committee-approval and information about keeping the data computerized and anonymous (29). The women accepted enrolment to the study when returning the questionnaires. The participants were also informed that the study is linked to the Cancer Registry of Norway and The Norwegian Cause of Death Registry (28). All data were anonymized before distribution to this project. Therefore, data used in this thesis cannot be traced back to any individuals. As a safety measure, the data has been saved in Onedrive with two-factor authentication login. The dataset received from the NOWAC study will be deleted after this project has ended.

4. Results

4.1 Population characteristics

After exclusion, 90 792 participants were included in the analyses. In each quartile of total fat intake, there was 22 698 women. Age slightly decreased as the intake of total fat increased. Participants in quartile 1(Q1) had a mean age of 52.8 years, while participants in quartile 4 (Q4) had a mean age of 51.2 years. Total energy intake increased from the group with the lowest intake of total fat to the group with the highest intake. In Q1 the energy intake was 4997 kJ, whereas for Q4 the intake was 9244 kJ. For LNYM, the lengths were almost identical, with approximately 33.5 years as average for all quartiles. Compared to the other quartiles, participants in Q4 had the highest percentage of underweight (2.0%) and lowest of participants with obesity (9.6%), but most reported to have normal weight (62.0%). Q4 also had the most participants who reported to be current smokers (33.6%) and compared to Q1 and Q3, a larger proportion in Q4 reported to have >13 years of education. Q4 had the most active participants with 17.6%, meanwhile in Q1, 15.3% was in the category with low physical activity. Still, 71.1-74.0% of all the participants were moderately active for all quartiles of total fat. Those who had the highest intake of total fat also had the highest intake of saturated fat (84.7%), monounsaturated fat (86.6%), polyunsaturated fat (69.1%), trans fat (65.2%) and cholesterol (65.4%).

Table 1. Descriptive statistics.

	Quartiles of total fat intake				
	Q1 (0-47.58g)	**Q2 (47.58g-60.40g)**	**Q3 (60.40-75.53 g)**	**Q4 (>75.53g)**	**Missing, n**
Participants, n	22 698	22 698	22 698	22 698	0
Age at baseline (y), mean (SD)	52.8 (6.9)	51.9 (6.5)	51.5 (6.4)	51.2 (6.2)	0
Total energy intake (kJ), mean (SD)	4997 (984)	6380 (846)	7434 (887)	9244 (1428)	0
LNYM (y), median (IQR)	33.8 (5.2)	33.7 (5.0)	33.6 (5.2)	33.3 (5.2)	1631
BMI (kg/m^2), %					0
Underweight	1.0	0.9	1.2	2.0	
Normal weight	52.0	55.6	59.2	62.0	
Overweight	33.9	32.5	29.3	26.4	
Obesity	13.0	10.9	10.3	9.5	
Education (y), %					0
0-9	30.0	25.7	23.9	24.2	
10-12	34.6	34.2	34.4	34.4	
>13	35.3	40.1	41.6	41.5	
Smoking, %					1378
Never	37.3	38.3	37.9	36.7	
Former	34.6	34.3	33.0	29.6	
Current	28.0	27.4	29.0	33.6	
Physical activity, %					5860
Low	15.3	12.8	11.9	11.1	
Moderate	71.1	74.0	73.6	71.3	
High	13.4	13.0	14.4	17.6	
Trans fatty acids (g/d), %					0
Q1 (0-0.8)	60.8	24.2	11.4	3.5	
Q2 (0.8-1.1)	31.4	36.9	22.0	9.6	
Q3 (1.1-1.5)	7.4	32.4	38.5	21.5	
Q4 (1.5<)	0.3	6.3	28.0	65.2	
Polyunsaturated fatty acids (g/d), %					0
Q1 (0-8.2)	73.7	20.9	4.5	0.8	
Q2 (8.2-10.7)	22.4	46.5	25.2	5.7	
Q3 (10.7-14.0)	3.8	27.8	44.0	24.3	
Q4 (>14.0)	0.1	4.5	26.2	69.1	
Monounsaturated fatty acids (g/d), %					0
Q1 (0-15.1)	87.6	12.2	0.0	0.0	
Q2 (15.1-19.0)	12.3	71.8	15.7	0.0	
Q3 (19.0-23.8)	0.0	15.7	70.9	13.3	
Q4 (>23.8)	0.0	0.0	13.2	86.6	
Saturated fatty acids (g/d), %					0
Q1 (0-18.9)	85.1	14.3	0.5	0.0	
Q2, (18.9-24.2)	14.8	66.9	17.7	0.6	
Q3 (24.2-30.6)	0.1	18.6	66.5	14.7	

Q4 (>30.6)	0.0	0.1	15.2	84.7	
Cholesterol (mg/d), %					0
Q1 (0-203.4)	65.5	24.6	8.2	1.5	
Q2 (203.4-254.5)	24.8	40.4	26.9	7.7	
Q3 (254.5-313.0)	7.98	26.9	39.7	25.3	
Q4 (>313.0)	1.6	7.8	25.0	65.4	

Abbreviations: BMI, body mass index; IQR, interquartile range, kJ: kilojoules, LNYM, lifetime number of years of menstruation; SD, standard deviation, y; years, Q; quartile.

4.2 Results from the statistical analyses

Results showed that the assumption of proportional hazards was met for all analyses, through visually inspection of the Schoenfeld's residual plots. The results from the analyses between the intake of fat and risk of EOC is presented in Table 2.

Table 2. Results from the analyses.

	Model 1					Model 2					Sensitivity analysis				
	Total n/cases	HR	p-value	95% CI	p-trend	Total n/cases	HR	p-value	95% CI	p-trend	Total n/cases	HR	p-value	95% CI	p-trend
Total fat	90 792/596					90 792/596					84 637/549				
Q1	22 698/159					22 698/159					20 836/139				
Q2	22 698/149	0.94	0.606	0.75-1.17		22 698/149	0.94	0.643	0.75-1.18		21 095/142	1.01	0.883	0.80-1.28	
Q3	22 698/132	0.84	0.145	0.66-1.06		22 698/132	0.84	0.165	0.67-1.06		21 124/124	0.89	0.381	0.70-1.14	
Q4	22 698/156	1.02	0.843	0.81-1.27	0.943	22 698 /156	1.02	0.796	0.82-1.28	0.896	21 172/144	1.06	0.591	0.84-1.34	0.739
Trans fatty acids	90 792/596					90 792/596					84 637/549				
Q1	22 698/130					22 698/130					21 156/114				
Q2	22 698/162	1.14	0.264	0.90-1.43		22 698/162	1.13	0.272	0.90-1.43		21 252/150	1.19	0.154	0.93-1.52	
Q3	22 698/151	1.02	0.838	0.81-1.29		22 698/151	1.02	0.845	0.80-1.29		21 162/141	1.08	0.520	0.84-1.38	
Q4	22 698/153	1.02	0.841	0.81-1.29	0.855	22 698/153	1.02	0.837	0.81-1.29	0.865	21 067/144	1.09	0.456	0.85-1.40	0.732
Polyunsaturated fatty acids	90 792/596					90 792/596					84 637/549				

Q1	22 698/161					22 698/161					20 833/146				
Q2	22 698/137	0.85	0.177	0.68-1.07		22 137/137	0.85	0.188	0.68-1.07		21 165/128	0.87	0.260	0.68-1.10	
Q3	22 698/145	0.91	0.465	0.73-1.15		22 698/145	0.92	0.477	0.73-1.15		21 242/135	0.93	0.554	0.73-1.17	
Q4	22 698/153	0.99	0.987	0.79-1.24	0.756	22 698/153	0.99	0.959	0.79-1.24	0.791	21 397/140	0.97	0.851	0.77-1.23	0.931
Monounsaturated fatty acids	90 792/596					90 792/596					84 636/549				
Q1	22 698/158					22 698/158					20 812/139				
Q2	22 698/154	0.98	0.880	0.78-1.22		22 698/154	0.99	0.931	0.79-1.23		21 219/144	1.03	0.771	0.81-1.30	
Q3	22 698/127	0.82	0.101	0.65-1.03		22 698/127	0.83	0.122	0.65-1.05		21 266/121	0.88	0.324	0.69-1.12	
Q4	22 698/157	1.06	0.586	0.85-1.32	0.781	22 698/157	1.07	0.517	0.86-1.34	0.701	21 340/145	1.10	0.392	0.87-1.39	0.551
Saturated fatty acids	90 792/596					90 792/596					84 637/549				
Q1	22 698/152					22 698/152					20 870/136				
Q2	22 698/152	0.99	0.992	0.79-1.25		22 698/152	1.00	0.975	0.80-1.25		21 277/140	1.01	0.900	0.80-1.28	
Q3	22 698/143	0.94	0.596	0.74-1.18		22 698/143	0.94	0.640	0.75-1.19		21 252/135	0.98	0.884	0.77-1.24	

Q4	22 698/149	0.99	0.949	0.79-1.24	0.871	22 698/149	0.99	0.995	0.79-1.25	0.918	21 238/138	1.01	0.883	0.80-1.29	0.933
Cholesterol	90 792/596					90 792/596					84 637/549				
Q1	22 698/157					22 698/157					20 845/140				
Q2	22 698/148	0.94	0.591	0.75-1.17		22 698/148	0.94	0.625	0.75-1.18		21 257/140	0.98	0.885	0.77-1.24	
Q3	22 698/136	0.87	0.239	0.69-1.09		22 698/136	0.87	0.266	0.69-1.10		21 226/129	0.91	0.477	0.72-1.16	
Q4	22 698/155	1.02	0.857	0.81-1.27	0.922	22 543/155	1.02	0.821	0.82-1.28	0.885	21 309/140	1.01	0.879	0.80-1.28	0.960

Abbrevations: n, number of participants, HR, Hazard ratio, CI, Confidence intervals

4.2.1 Total fat

4.2.1.1 Model 1

Compared to the women with the lowest intake of total fat (Q1), the other quartiles were not significant associated with the risk of EOC after adjusting for age. All p-values were >0.05. HR for quartile 2 (Q2) was 0.94 (95% CI: 0.75-1.17) and HR for quartile (Q3) was 0.84 (95% CI: 0.66-1.06). In contrast to Q2 and Q3, the HR for Q4 was >1.00 (HR= 1.02, 95% CI: 0.81-1.27). The p-trend for model 1 was also not significant (p=0.943).

4.2.1.2 Model 2

After adjusting for education, the HRs remained the same as in model 1 when compared to Q1. 95% CIs changed slightly to Q2: 0.75-1.18, Q3: 0.67-1.06 and Q4: 0.82-1.28. No significant p-values were observed in model 2. P-trend across the four quartiles in model 2 was still not significant (p-trend = 0.896).

4.2.1.3 Sensitivity analysis

When further adjusting for history of breast cancer in addition to education and age, the HRs increased to 1.01 (CI: 0.80-1.28), 0.89 (CI: 0.70-1.14) and 1.06 (CI: 0.84-1.34) when comparing to Q1. Still, all associations were non-significant as the p-values were > 0.05, including p-trend (0.739).

4.2.2 Trans-fat

4.2.2.1 Model 1

In the age adjusted analyses, there were no significant p-values for trans fatty acids when comparing Q2-Q4 to Q1. HR for Q2 was 1.14 (CI: 0.90-1.43) while both Q3 and Q4 had a lower HR of 1.02 with 95% CIs of 0.81-1.29. P-trend across the quartiles was 0.855.

4.2.2.2 Model 2

After further adjusting for education at baseline, the HRs and p-values from model 2 was mostly unchanged from model 1, and the p-values still shows non-significance. Specifically, HR for Q2 was 1.13 (95% CI: 0.90-1.43) while HRs for Q3 and Q4 still remained at 1.02 (Q3 95% CI: 0.80-1.29 and Q4 95% CI: 0.81-1.29) when compared to Q1. P-trend changed to 0.865, meaning still no significance.

4.2.2.3 Sensitivity analysis

HRs for after adjusting for age, education at baseline and family history of breast cancer concerning trans fatty acids were 1.19 for Q2 (95% CI: 0.93-1.52), 1.08 for Q3 (95% CI: 0.84-1.38) and 1.09 for Q4 (95% CI: 0.85-1.40), when compared to Q1. Further, all p-values were non-significant and p-trend across quartiles was 0.732.

4.2.3 Polyunsaturated fat

4.2.3.1 Model 1

In the age adjusted model, all p-values showed non-significance for the association between polyunsaturated fatty acids and risk of EOC, including p-trend (0.756). All HRs was <1.0 when Q2-Q3 were compared to Q1 in the analyses. The HR and 95% CI for Q2 was 0.85 (0.68-1.07), for Q3 it was 0.91 (0.73-1.15) and lastly, for Q4, the HR with 95% CI was 0.99 (0.79-1.24).

4.2.3.2 Model 2

HRs and 95% CIs for model 2 were unchanged by the addition of education as a confounder when comparing Q2-Q4 with Q1. Specifically, HR for Q2 was 0.85 (95% CI: 0.68-1.07), Q3 was 0.92 (95% CI: 0.73-1.15) and Q4 was 0.99 (95% CI: 0.79-1.24). P-trend across the quartiles was 0.791. No significant p-values were observed.

4.2.3.3 Sensitivity analysis

When further adjusting for history of breast cancer in addition to age and education, the HRs for Q2, Q3 and Q4 compared to Q1 were slightly changed, respectively 0.87 (95% CI: 0.68-1.10), 0.93 (95% CI: 0.73-1.17) and 0.97 (95% CI: 0.77-1.23). P-trend was 0.931 and no p-values were significant.

4.2.4 Monounsaturated fatty acids

4.2.4.1 Model 1

In the age-adjusted analysis for monounsaturated fatty acids, the HRs were 0.98 (95% CI: 0.78-1.22), 0.82 (95% CI: 0.65-1.03) and 1.06 (95% CI: 0.85-1.32) for Q2, Q3 and Q4 compared to Q1. No p-values showed any significance, and p-trend across quartiles was 0.781.

4.2.4.2 Model 2

When additionally adjusting for education, HRs for model 2 were marginally increased unchanged to 0.99 (Q2), 0.83 (Q3) and 1.07 (Q4). 95% CIs were also unchanged to 0.79-1.23 (Q2), 0.65-1.05 (Q3) and 0.86-1.34 (Q4). P-trend showed a value of 0.701 and no significant p-values were observed.

4.2.4.3 Sensitivity analysis

When adjusting for family history of breast cancer in addition to age and education, the HRs changed to 1.03 for Q2 (95% CI: 0.81-1.30), 0.88 for Q3 (95% CI: 0.69-1.12) and 1.10 for Q4 (95% CI: 0.87-1.39). All p-values were non-significant and p-trend across quartiles was 0.551.

4.2.5 Saturated fatty acids

4.2.5.1 Model 1

In the age-adjusted analysis, all p-values showed non-significance, and all HRs was <1.0. HR and 95% CIs for Q2 was 0.99 (0.79-1.25), for Q3 it was 0.94 (0.74-1.18) and lastly for Q4 it was 0.99 (0.79-1.24) when compared to Q1. P-trend was 0.871 across quartiles.

4.2.5.2 Model 2

When further adjusting for education, model 2 for saturated fatty acids showed HRs for Q2 of 1.00 (95% CI: 0.80-1.25), and further 0.94 for Q3 (95% CI: 0.75-1.19). Q4 had an HR of 0.99 with 95% CI: 0.79-1.25. There were no p-values < 0.05 and p-trend was 0.918.

4.2.5.3 Sensitivity analysis

When further adjusting for history of breast cancer in addition to education and age, the HRs changed to 1.01 (95% CI: 0.80-1.28), 0.98 (95% CI: 0.77-1.24) and 1.01 (95% CI: 0.80-1.29) for Q2, Q3 and Q4 respectively, when compared to Q1. The analyses in the sensitivity analysis only showed non-significant p-values as in model 1 and 2. P-trend was 0.933 across quartiles.

4.2.6 Cholesterol

4.2.6.1 Model 1

In model 1 when only adjusting for age, the HRs for Q2 was 0.94 (95% CI: 0.75-1.17), 0.87 for Q3 (95% CI: 0.69-1.09) and 1.02 for Q4 (95% CI: 0.81-1.27) when compared to Q1. All p-values were >0.05 and p-trend was 0.922.

4.2.6.2 Model 2

HRs for model 2 were unaffected by adding education to the analysis, and the 95% CIs changed only slightly to 0.75-1.18 (Q2), 0.69-1.10 (Q3) and 0.82-1.28 (Q4) when comparing to Q1. No significant p-values were observed in the analyses, and p-trend was 0.885.

4.2.6.3 Sensitivity analysis

The sensitivity analysis for cholesterol when adjusting for age, education and family history of breast cancer showed HRs and 95% CIs of 0.98 (0.77-1.24) for Q2, 0.91 (0.72-1.16) for Q3 and 1.01 (0.80-1.28) for Q4, when compared to Q1. All p-values showed a value greater than 0.05 and p-trend was 0.960.

5. Discussion

In this thesis, the purpose was to evaluate the possible association between the intake of total fat and different types of fat and the risk of developing epithelial ovarian cancer for women in Norway. By using Cox regression and data from the NOWAC study, the analyses of total fat, trans fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, and cholesterol were analysed in three different models. Model 1 was adjusted for age, meanwhile model 2 was additionally adjusted for education. The sensitivity analyses were also adjusted for family history of breast cancer. When comparing the highest quartile with the lowest quartile of the different types of fat, the HRs of developing EOC when adjusted for age and education were larger than 1.00 for total fat, trans fatty acids, monounsaturated fatty acids, and cholesterol, while HRs was less than 1.00 for polyunsaturated fatty acids and, saturated fatty acids. However, none of the associations investigated in this thesis were statistically significant. This implies that the intake of total fat, different fatty acids or cholesterol is not associated with the risk of developing EOC in middle-aged Norwegian women.

5.1 Discussion of results

5.1.1 Total fat

The analyses for total fat showed no p-values <0.05 and all 95% Cis were wide for all models. The wider the CI, the less certain the results are and may not be precise for the populations in the study. Except for Q2 in model 3, all HRs for Q2 and Q3 in all three models had HRs <1.0, however Q4 was >1.0 (1.02, 1.02 and 1.06), and one could think that a high intake of total fat

could be associated with the risk of EOC. However, since no p-values were ever close to being <0.05, no conclusion can be made.

Other studies have found varying results. Qui et al (24) conducted a meta-analysis in 2016 pooling data from six cohorts, and found no linkage between self-reported consumption of total fat and risk of EOC (RR: 1.10, 95% CI: 0.97-1.24). However, Sadeghi et al (41) reported an increased risk of EOC when the intake exceeded 30 g/d in their non-linear dose-response association. Also, in their linear dose-response meta-analysis the findings showed an increased risk of EOC of 2% for every additional 10 g of total fat intake daily (RR: 1.02, 95% CI: 1.01-1.02) (41).

There are several suggested mechanisms for how total fat intake can affect the risk of EOC. A high consumption of fat can lead to several negative health outcomes such as obesity, insulin resistance, hyperinsulinemia and cause high levels of growth factor-1 receptor. All of these can induce androgen steroidogenesis and result in EOC (18). There is also evidence regarding stimulation of the secretion of oestrogen from the ovaries, when having a high intake of total fat, which further can lead to EOC (19).

5.1.2 Trans fatty acids

For trans-fat, no p-values <0.05 were observed, including p-trend for all models and all CI were wide. Hence, the results indicate that the intake of trans fatty acids have no association with EOC risk for Norwegian women. In addition, when Cis are wide and contains the value 1.0, means the results are not significant. Interestingly, the HRs did not increase with increasing intake of trans fatty acids. Instead, the highest HR was observed in Q2 for all models. One might anticipate that the risk of EOC increased with increasing intake, since the intake of trans fatty acids is higher in the general population then what is recommended (15). Sadeghi et al (41) found an association between trans-fat and EOC in their linear dose-response meta-analysis of four studies. The risk of EOC increased with 2% for each additional increase of 0.5 g trans-fat daily (RR: 1.02, CI: 1.01-1.03). In the results from this thesis, the highest risk was observed in Q2, and it did not find a linear association like Sadeghi et al (41).

A meta-analysis conducted by Hou et al (25) found similar results as in this thesis. They observed a RR of 1.15 (95% CI: 0.98-1.36) for the association between the trans-fat intake and EOC risk. Contrary to the results in this thesis, other studies have found associations

between trans fatty acids and risk of EOC (16, 24, 25). In a meta-analysis by Qiu et al (24), the authors found significant (p=0.002), positive associations between trans fatty acids and EOC risk (Overall RR: 1.25, 95% CI: 1.08-1.44).

The differing results can have several possible explanations, such as different study designs and different factors adjusted for in the analyses. Merritt et al (26) was one of the studies included in the meta-analysis by Qui et al (24). They found an 30% increased risk of EOC when comparing participants with a high intake of trans fatty acids to participants with a low intake (OR=1.30, 95% CI: 1.08-1.57, p-trend= 0.002). Merritt et al (26) is a case-control study and used logistic regression as their statistical analysis. A prospective cohort study, like the NOWAC study, is less likely to have issues with selection bias compared to a case-control study. Case-control studies are dependent on a control group which is comparable to the case group, meanwhile the participants in a cohort study are enrolled before diagnosis. Case-control studies often encounter "recall bias," where individuals with the disease may recall differently or more accurately than the controls, which are not affected by the disease (42).

Both study design can be used to study rare diseases, such as EOC, but a case-control study cannot estimate the incidence of disease, because there is no information on how many in a population that were exposed or unexposed. Concerning confounders, Merritt et al (26) used other confounders to adjust in their analyses; age, study center, study phase, number of pregnancies, use of oral contraceptives and family history of ovarian cancer tubal ligation, which may have caused the different results.

Trans-fat may be the type of fat that is most often associated with negative outcomes and risks of disease. Trans fatty acids appear to trigger inflammation in the human body and have a negative impact on endothelial function. Further, abundant evidence suggests that chronic inflammation is linked to the process of carcinogenesis and therefore increase the EOC risk (20, 21).

5.1.3 Polyunsaturated fatty acids

All HRs for polyunsaturated fatty acids was below 1.0 when comparing to the women with the lowest intake. Meanwhile, all p-values were non-significant, and 95% Cis were somewhat wide. The wide 95% CI can indicate an uncertain estimate, in this case, HR. Therefore, the findings in this thesis does not suggest a link between the intake of polyunsaturated fatty acids and developing EOC. Also, in the scoping review from NNR 2023 (16), no association was

found between polyunsaturated fatty acids and the risk EOC in any of the included studies. However, only two of the studies in the scoping review looked at polyunsaturated fatty acids. In a meta-analysis by Qiu et al (24), included in the NNR 2023 review, with eight case-control and five cohort studies, the overall RR was 0.97 (95% CI: 0.86-1.10). Meanwhile p-value was 0.760 which suggested no association. The other study in the scoping review which included polyunsaturated fatty acids, Sadeghi et al (41), observed in their meta-analysis a pooled RR of 1.0 (95% CI: 0.99-1.02) for 2.5 g/day increment of polyunsaturated fatty acids. A RR of 1.0 means there is no increasing or decreasing risk of developing EOC, meaning there is no association. Also, the 95% CI contains the value 1.0 and is narrow, supporting the RR in the meta-analysis.

Animal studies have shown that polyunsaturated fatty acids, especially long-chain n-3 polyunsaturated fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can suppress the development of several types of cancer (43). The theory behind the mechanism suggests that DHA can induce apoptosis and inhibit tumour proliferation, invasion, and metastasis (44). In a Mendelian randomization study by Zhang et al (44), the plasma DHA levels and risk of ovarian cancer was assessed in populations in Europe and Asia. For the European population the findings showed a causal relationship between high levels on DHA and risk of ovarian cancer. When doing subgroup analysis by histological type of ovarian cancer, the association was the strongest for endometrioid ovarian cancer, which is a type of EOC. Thus, suggesting that the intake of polyunsaturated fatty acids may affect subtypes of EOC differently.

5.1.4 Monounsaturated fatty acids

The results for monounsaturated fatty acids showed no p-values <0.05 and had wide 95% Cis. All HRs for Q3 could indicate a protective factor. HRs were 0.82 (Model 1), 0.83 (Model 2) and 0.88 (Sensitivity-analysis). Contrarily, all HRs for Q4 were >1.0, implying that a high intake of monounsaturated fatty acids could increase a woman's' risk of EOC. But as earlier stated, the p-values for these HRs are way above 0.05, and therefore are not significant. Like this study, other studies have also found no associations between monounsaturated fatty acids and risk of EOC (24, 25, 45). In contrast of these findings, Sadeghi et al (41) found a nonlinearly increasing association when the intake of monounsaturated fatty acids exceeded 25 g/day. This cutoff is comparable with the results in this thesis, as the intake of monounsaturated fatty acids in Q4 was >23.8 g/d.

Monounsaturated fatty acids are known to decrease the LDL cholesterol and increase the HDL cholesterol in the blood, and in this way also effects the risk of EOC through the risks of having a high cholesterol levels (17).

5.1.5 Saturated fatty acids

Concerning saturated fatty acids, all HRs were close to 1.0 (ranging from 0.94-1.01) and no p-values <0.05 were observed, including p-trend for all models. In addition, all CI were wide. Therefore, the results indicate that there is no association between the intake of trans fatty acids and EOC risk among Norwegian women.

Unlike the findings in this thesis, other studies have found a significant association between saturated fatty acids and risk of EOC (16, 22, 24). Qui et al (24) studied six case-control and six cohort studies and found that a higher consumption of saturated fat increased the risk of EOC with 12% (Overall RR: 1.12 (95% CI: 1.02-1.22), p=0.014). Merritt et al (22) found no association when studying the EPIC study alone, however in their meta-analyses of the EPIC study and NLCS combined there was a 21% higher risk of EOC when consuming a high intake of saturated fatty acids compared to a low intake (Overall HR: 1.21, 95% CI: 1.04-1.41). Sadeghi et al (41) reported a nonlinearly association increased risk if the intake exceeds 25 g/day. Hou et al (25) only found non-significant results for saturated fatty acids in their meta-analysis and concluded that there is not enough evidence to establish a linkage between dietary fatty acids in general and risk of EOC. One suggested mechanism for the development of EOC is increased oestrogen production in the ovaries, and that a high intake of saturated fatty acids can stimulate this production (19).

5.1.6 Cholesterol

For cholesterol, all HRs was below 1.0 when compared to a low intake. Still, all p-values were non-significant, and 95% CIs are wide, meaning the findings in this thesis did not find evidence for a linkage between cholesterol and risk of EOC. Sadeghi et al (41) found a marginal association between intake of cholesterol and risk of EOC through their linear dose-response meta-analysis with seven studies. The unit of exposure was set as 50 mg/d, and the findings stated an increased risk of 1% for every additional unit of exposure. Contrarily, Genkinger et al (45) did not find an association in their pooled analysis. The mechanism behind the suggested theory that cholesterol may contribute to increased risk of EOC is the link between cholesterol being a precursor to the synthesis of steroid hormones, including

oestrogen. On another note, several of the studies mentioned in this thesis studying intake of fat and risk of EOC did not include cholesterol.

5.2 Strengths & limitations

5.2.1 Prospective study design

When looking at study design, the NOWAC study is a prospective cohort study invented to study the relationship between different lifestyle factors, exposures and the risk of developing cancer(28). These types of studies are not conducting any experiments or drug-testing, but the aim is to observe participants in their everyday environments and life choices. In the field of research and epidemiology, randomized control trials are considered the gold-standard for analysing causal associations between exposure and outcome. However, when studying cancer, a study design with long follow-up period and big populations that are easier and cheaper to conduct is recommended. For cancer, which we know evolves over time, the follow-up time is an important aspect to evaluate in the analyses. The longest follow-up time is this thesis was 29.6 years. When the women in the NOWAC study have answered FFQs before developing cancer, the exposure is already assessed before the outcome occurs. When assessing exposures after the outcome, is it prone to more bias (46). This is a strength in the NOWAC study, although longitudinally studies also have the disadvantage having follow-up for a long period of time, meaning many more exposures and factors could affect the outcome.

5.2.2 Sample size

When studying a small cancer type with few cases, it is important to have many participants. Out of 90 792 participants, 596 cases of EOC were reported. For the analyses to have sufficient statistical power, its important with large sampling size to control the risk of false-negative or false-positive findings (47). To secure an even bigger sample size, imputation could have been done, but were considered to be beyond the scope of this thesis.

5.2.3 External validity

A common bias for health research is the selection bias that may occur at enrolment in the study. For the NOWAC study, a strength is the fact that women from all over the country is randomly selected through the National Registry. Meaning it could better describe the whole population of Norway, instead of only subgroups. However, sampling bias may appear when women who has interest in health research more often accept the enrolment to these studies,

than those who are not interested. Also called the healthy volunteer effect. Considered the participants in the NOWAC study has a somewhat higher educational level, If the dataset is biased with more highly educated women than the average population is, it could also affect other variables. In that case, the findings may only be describing for women who are highly educated. Highly educated women are less prone to smoke, have a high BMI, be physical unactive and in general live a healthier lifestyle than average.

5.2.4 FFQ

The dietary assessment used in the NOWAQ study is the FFQ, which is a common tool for collecting information on a person's intake, when doing nutritional research. FFQs are inexpensive, easy to send out and collect, and can give a study a lot of data without being too time-consuming (38). Still, FFQs could also have its disadvantages. Seeing that FFQs are based on questions about a participants frequently intake the last year, it relies on the fact that the participants remember their intake over time correctly. Also, not only what they eat, but also the quantities and portion sizes. Under- and overestimating food intake is widely known in nutritional research and can result in bias. Some food groups can also be trickier to remember than others. When asking about seasonal intake like berries for example, one may not remember how often eating strawberries during summer, when answering the FFQ in the winter.

Another common, possible disadvantage of a FFQ can be social desirability bias. Diet and food intake is personal to participants and can in many cases lead to hiding their true intake. Often not intentionally, participants tend to report an intake closer to the recommendations or what is viewed as more socially acceptable, than what their true intake is. Intake of alcohol is often underreported, as well as other foods or drinks considered unhealthy. Therefore, it also needs to be taken into considerations that the fat intake reported from the FFQs are inaccurate. Especially the trans fatty acids and saturated fatty acids could be underreported, because of this social desirability bias.

Hence its large sampling size, based on population from a whole country, it is important to remember that the FFQ is based on a Norwegian diet, cuisine and culture. The FFQs are designed to cover and summarize what Norwegians may consume. Considering the intake of fat was the diet question of interest, one may take into consideration the fat sources in Norway, and compare them to other countries, and see if the results therefore are representative. Other countries may have different sources of fat, and different distribution of

subgroups of fatty acids. However, there are many sources to fat in different food items, and it may be the food items, and not only fat that affects our health.

Questions about the use of cod liver oil capsules were included in the FFQs. However, information on the use of these supplements (type and frequency) was not calculated into daily intake and thus not included in the variable for intake of polyunsaturated fatty acids. Therefore, the intake of polyunsaturated fatty acids among the participants could be higher than the intake reported in this thesis.

5.2.5 Confounding factors

The method to find the confounding variables was to construct a DAG. When conducting a DAG, all assumptions are based on earlier research to determine which factors affect each other. Therefore, if any of these conjectures are wrong, the DAG and the confounding variables will be inaccurate. Some associations were strongly evidenced, while some were limited researched. For several associations, it was also debateable which way the factors affected each other. Also, some choices were made based on the study population, such as not linking age to height. Age effects your height if you are 6 vs 25 years old, but when taking into consideration the study population being 41-76 years old, the link is weak.

When doing research for this thesis, the literature showed that a family history of breast- and ovarian cancer could increase the risk of developing EOC. Considering the NOWAC study only had information on family history of breast cancer, and not family history of EOC. Thus, family history of EOC could not be adjusted for in the statistical analyses. Breast cancer and ovarian cancer are linked due to them both being connected to the BRCA1 and BRCA2 genes (48).

A limitation of this thesis is that an additional sensitivity analysis was planned to be conducted where the participants which developed cancer within 2 years after enrolment was excluded. This would reduce the possibility of reverse confounding, where some participants may have changed their diets due to disease. Specifically, cancer at an early stage may affect the diet, but may not yet have been diagnosed.

6. Conclusion

In this thesis, no association between the intake of total fat, trans fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids and cholesterol and risk of developing EOC among the participants in the NOWAC study was observed. However, there are inconclusive results reported from other studies, and more research is needed on this topic.

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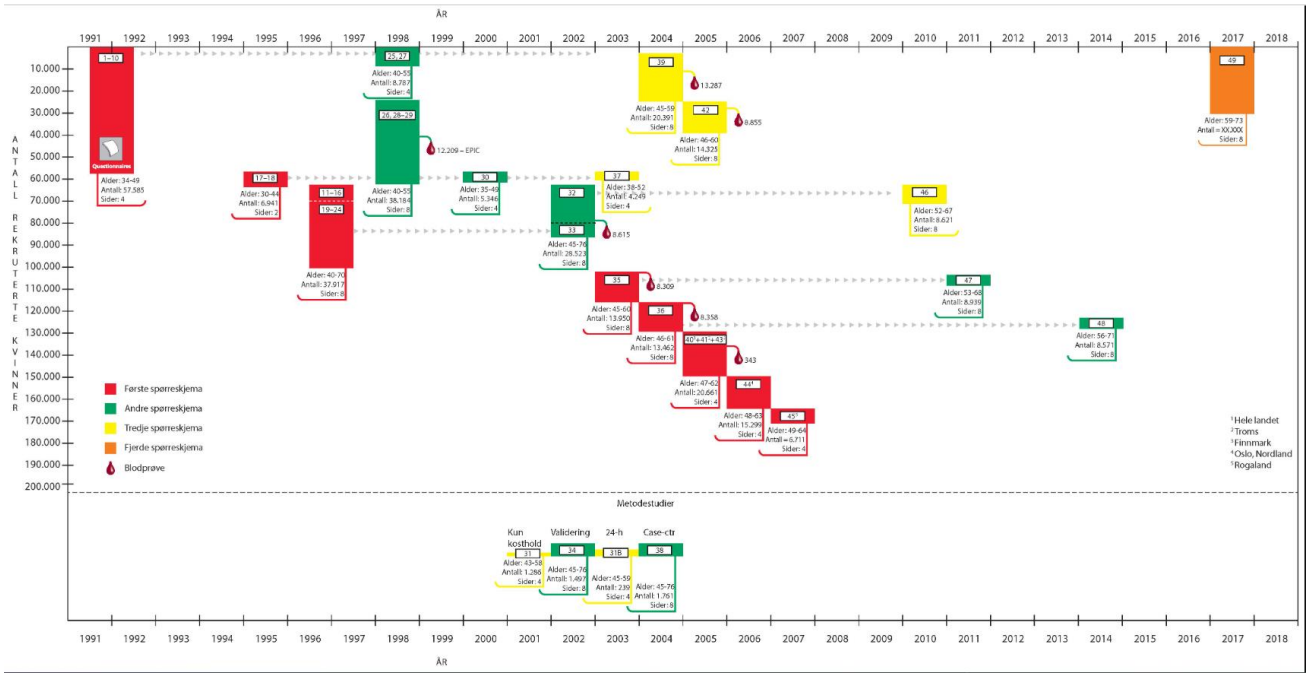
Appendix 1



Visual representation of the total NOWAC-cohort. 49 series from 1991-2017 (28).
Red: First questionnaire
Green: Second questionnaire
Yellow: Third questionnaire
Orange: Fourth questionnaire