Ovarian Cancer Risk in Relation to Blood Cholesterol and Triglycerides

Britton Trabert[1,2,3], Cassandra A. Hathaway[4], Megan S. Rice[5], Eric B. Rimm[6,7,8], Patrick M. Sluss[9], Kathryn L. Terry[7,10], Oana A. Zeleznik[8], and Shelley S. Tworoger[4,7]

ABSTRACT

Background: The association between circulating cholesterol and triglyceride levels and ovarian cancer risk remains unclear.

Methods: We prospectively evaluated the association between cholesterol [total, low-density lipoprotein (LDL-C), and high-density lipoprotein (HDL-C)] and triglycerides and ovarian cancer incidence in a case–control study nested in the Nurses' Health Study (NHS) and NHSII cohorts and a longitudinal analysis in the UK Biobank.

Results: A total of 290 epithelial ovarian cancer cases in the NHS/NHSII and 551 cases in UK Biobank were diagnosed after blood collection. We observed a reduced ovarian cancer risk comparing the top to bottom quartile of total cholesterol [meta-analysis relative risk (95% confidence interval): 0.81 (0.65–1.01), P_{trend} 0.06], with no heterogeneity across studies ($P_{heterogeneity} = 0.74$). Overall, no clear patterns were observed for HDL-C, LDL-C, or triglycerides and ovarian cancer risk. Comparing triglyceride levels at clinically relevant cut-off points (>200 vs. ≤200 mg/dL) for cases diagnosed more than 2 years after blood draw saw a positive relationship with risk [1.57 (1.03–2.42); $P_{heterogeneity} = 0.003$]. Results were similar by serous/non-serous histotype, menopausal status/hormone use, and body mass index.

Conclusions: Data from two large cohorts in the United States and United Kingdom suggest that total cholesterol levels may be inversely associated with ovarian cancer risk, while triglycerides may be positively associated with risk when assessed at least 2 years before diagnosis, albeit both associations were modest.

Impact: This analysis of two large prospective studies suggests that circulating lipid levels are not strongly associated with ovarian cancer risk. The positive triglyceride–ovarian cancer association warrants further evaluation.

Introduction

High total cholesterol is a well-established risk factor for coronary heart disease and stroke (1, 2). However, the relationship between total cholesterol and cancer risk remains uncertain. Cholesterol is a precursor to several biochemical pathways, including the synthesis of vitamin D and steroid hormones, and is thought to be involved in the etiology of certain cancers (3, 4). The associations of blood cholesterol and triglyceride levels with cancer outcomes (including ovarian cancer) vary considerably across different studies (5).

In prospective studies, associations with ovarian cancer across extreme quantiles of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), and triglycerides were generally null, although effect estimates differed across United States and European populations (6–10). Notably, there was some support for an association between higher total cholesterol with increased risk of serous and mucinous tumors in a study conducted in northern Europe, but no evidence of an association with endometrioid tumors (8). Using an alternate approach of evaluating cut-off points for hyperlipidemia, a small study in Taiwan, reported null ovarian cancer associations with high cholesterol (>200 mg/dL) and high triglycerides (>150 mg/dL), while a larger study in China reported an approximately 50% lower risk for those with high HDL-C (>1.0 vs. ≤1.0 mmol/L) and a nearly 3-fold higher risk with high triglycerides (>1.7 vs. ≤1.7 mmol/L; ref. 11).

Furthermore, there is conflicting evidence evaluating various aspects of metabolic risk profiles based on medical record report of dyslipidemia or hyperlipidemia. Results of these studies range from a 36% reduction in ovarian cancer risk with a hyperlipidemia diagnosis in Italy (12) to no association in studies evaluating dyslipidemia in the United Kingdom (13) to an increased risk of high-grade serous and endometrioid ovarian cancer with high triglycerides in a SEER-Medicare linkage analysis with 16,580 cases in the United States (14).

Given the inconsistent associations in the studies published to date, the goal of the current study was to evaluate circulating levels of cholesterol (total, LDL-C, HDL-C) and triglycerides with ovarian cancer risk across two study populations, the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII) cohorts in the United States and the UK Biobank.

[1]Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, Maryland. [2]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, Utah. [3]Cancer Control and Population Sciences Research Program, Huntsman Cancer Institute, Salt Lake City, Utah. [4]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida. [5]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [6]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [7]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [8]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts. [9]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [10]Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Britton Trabert, Division of Cancer Epidemiology and Genetics, NCI, 9609 Medical Center Drive, Bethesda, MD 20892-9768. E-mail: britton.trabert@nih.gov

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Materials and Methods

Study populations

This analysis was based on data from case–control studies nested in the NHS and NHSII and a prospective cohort study in the UK Biobank. As previously described, the NHS was established in 1976 among 121,700 U.S. female nurses ages 30–55 years, and NHSII was established in 1989 among 116,429 female nurses ages 25–42 years (15, 16). Participants have been followed every other year by questionnaire to update information on exposures, including putative ovarian cancer risk factors, and disease diagnoses in both cohorts. Between 1989 and 1990, 32,826 NHS participants donated blood samples and completed a short questionnaire, including age, fasting status, menopausal status, medication use, and time and date of blood draw. Briefly, participants arranged to have their blood drawn and shipped on ice to a central laboratory via courier where it was processed and separated into plasma, red blood cell, and white blood cell components. In 2010, follow-up of the NHS blood study cohort was 87.5%. Similarly, between 1996 and 1999, 29,611 NHSII participants donated blood samples and completed a short questionnaire. Premenopausal participants ($n = 18{,}521$) who had not taken exogenous hormones, been pregnant, or lactated within the 6 months prior to blood draw provided a timed blood sample drawn 7–9 days before the anticipated start of their next menstrual cycle (luteal phase). Other women ($n = 11{,}090$) provided a single untimed blood sample. Following the same protocols as the NHS samples, NHSII samples were shipped to a central laboratory and processed. In 2009, follow-up of the NHSII blood study cohort was 95%.

As previously described, the UK Biobank is a population-based cohort study that invited more than 9 million individuals from across the UK to participate (17, 18). Under an approved protocol, we accessed baseline demographic, lifestyle, and biomarker data from the cohort. Briefly, the study mailed invitations to individuals ages 40–69 years old in the National Health Service who resided within 40 km of 22 assessment centers across the United Kingdom. In total, 503,317 individuals visited assessment centers between 2006 and 2010; answered comprehensive questionnaires via touchscreen providing information on known ovarian cancer risk factors; received physical examinations; and provided biological samples. Blood samples were collected at the assessment centers, minimally processed and shipped within 24 hours of collection for additional processing and storage at UK Biobank's centralized automated laboratory (19). Information about blood draw characteristics (e.g., date and time of collection) were recorded. Data from 502,528 participants were available for our study. We excluded participants identifying as male ($n = 229{,}134$), who withdrew consent or emigrated from the United Kingdom [$n = 688$ (22 withdrew after enrolling)], with prevalent cancer ($n = 17{,}777$), bilateral salpingo-oophorectomy at least a year before cancer diagnosis ($n = 31$), missing/implausible follow-up ($n = 38{,}269$), or missing information on serum cholesterol and/or triglyceride measurements ($n = 35{,}989$), leaving an analytic cohort of 180,640 female participants. Follow-up time was counted from the date of assessment center visit in 2006 until the date of cancer diagnosis, death, or the end of follow-up (i.e., March 31, 2015, for England and Wales and August 31, 2014, for Scotland), whichever came first.

Case ascertainment

Eligible cases had no history of cancer, except non-melanoma skin cancer, before blood collection and were diagnosed with ovarian or peritoneal cancer between blood draw and June 1, 2010 (NHS), or June 1, 2009 (NHSII). A total of 290 epithelial ovarian or peritoneal cancers (240 in NHS and 50 in NHSII) with plasma were confirmed by medical record review. Cases were matched to one control who was alive and had no history of oophorectomy at the time of the case diagnosis separately for each cohort. Matching factors included menopausal status at blood draw and diagnosis (premenopausal, postmenopausal, or unknown) and hormone therapy (HT) use among postmenopausal women (yes/no) at blood draw, age (±1 year), time of day of blood draw (2am–7am, 8am–1pm, 2pm—1am), and fasting status [>8 hours (fasting) or ≤8 hours (non-fasting)]. NHSII cases were additionally matched on timing of the blood draw within the menstrual cycle (date of next menstrual cycle minus date of blood draw, ±1 day) as applicable. In the UK Biobank, cancer cases during follow-up were identified via Health and Social Care Information Centre for participants residing in England and Wales and through the National Health Service for those residing in Scotland. Cancers were classified using the International Classification of Diseases, tenth revision (ICD-10), and included ovary (C569) and fallopian tube (C570) tumors.

Ethical approval

The NHS/NHSII studies were approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital (Boston, MA). Participant return of a baseline questionnaire and subsequent blood sample was considered to imply informed consent. The NHS study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital (Boston, MA) and Harvard T.H. Chan School of Public Health (Boston, MA), and those of participating registries as required. The UK Biobank study was approved by the National Information Governance Board for Health and Social Care and the NHS North West Multicenter Research Ethics Committee. Written consent was obtained from all participants, which was conducted in accordance with the principles of the Declaration of Helsinki.

Laboratory assays

The NHS and NHSII heparinized plasma samples were assayed for total cholesterol, HDL-C, direct LDL-C, and triglycerides using an ARCHITECT ci8200 analyzer (Abbott Diagnostics). High-sensitivity C-reactive protein (CRP) levels were measured via a validated immunoturbidometric method (Denka Seiken; ref. 20). Matched case–control sets were assayed together in the same batch. Coefficients of variation (CV) calculated from blinded replicate samples randomly interspersed with participant samples were ≤2% across all assay batches. These biomarkers are stable within person over time, with intraclass correlations over 1 year of 0.60–0.84 (21). In the UK Biobank, assays were performed at a dedicated central laboratory between 2014 and 2017, including measurement of serum total cholesterol, HDL-C, direct LDL-C, and triglycerides (Beckman Coulter AU5800). High-sensitivity CRP was measured by immunoturbidimetric analysis (Beckman Coulter AU5800). CVs for the UK Biobank assays were ≤3% across all assay batches (https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/serum_biochemistry.pdf).

Statistical analysis

Quartile cut-off points (in controls in NHS/NHSII and in the full population in UK Biobank) largely overlapped, thus we used the cut-off points derived from NHS/NHSII controls to enhance comparability across populations. For comparison with other studies, we also utilized clinical cut-off points for each marker representing hyperlipidemia as follows: total cholesterol > 240 mg/dL, LDL-C>160 mg/dL, HDL-C<40 mg/dL, and triglycerides > 200 mg/dL. In the NHS/NHSII, odds ratios (OR) and 95% confidence intervals (95% CI) were

determined using unconditional logistic regression comparing quartiles of biomarker concentrations. In the UK Biobank, HRs and 95% CI were determined using Cox proportional hazards models. Models in both populations were adjusted for age (continuous), blood draw time (2am–7am, 8am–1pm, 2pm–1am), menopausal status/HT use (premenopausal, postmenopausal/no HT use, postmenopausal/HT use, unknown), fasting status (no, yes), and *a priori* selected potential confounding factors, including oral contraceptive use (never, <1, 1–<5, 5+ years), parity (nulliparous, 1, 2, 3, 4+ births), body mass index (BMI; <25, 25–<30, 30–<35, ≥35 kg/m^2), tubal ligation (no, yes), hysterectomy (no, yes), and unilateral oophorectomy (no, yes). NHS/NHSII analyses were additionally adjusted for cohort (NHS, NHSII). We further evaluated models mutually adjusting for quartiles of HDL-C, LDL-C, and triglycerides and models adjusted for circulating CRP, a known inflammatory risk marker for ovarian cancer (22). Trend tests were assessed using a Wald test of the ordinal quartile variable. Relative risk (RR) estimates across studies were estimated using fixed-effects meta-analysis.

In secondary analyses, we stratified cases by time between blood draw and diagnosis [≤2 (n = 34 NHS/NHSII; 118 UK Biobank) vs. >2 years (n = 256 NHS/NHSII; 433 UK Biobank)] to evaluate the potential influence of undiagnosed cancers on associations (23). We further evaluated associations stratified by serous (n = 224 NHS/NHSII; 401 UK Biobank) and non-serous cases (n = 84 NHS/NHSII; 150 UK Biobank); cases with other histotypes (i.e., mucinous, clear cell, endometrioid) were too few to evaluate separately. Effect estimates by case characteristics (time between blood draw and diagnosis and serous/non-serous histotype) were estimated using multivariable adjusted polytomous logistic regression in NHS/NHSII and competing risk Cox models in the UK Biobank (24). We examined effect modification by categories of menopausal status/HT use and overweight/non-overweight BMI in stratified models. An alpha = 0.05 was used for all statistical tests and statistical analyses were conducted in SAS (V9.4) and meta-analyses in Stata (V16).

Data availability

Further information including the procedures to obtain and access data from the Nurses' Health Studies is described at https://www.nurseshealthstudy.org/researchers (contact email: nhsaccess@channing. harvard.edu) and from the UK Biobank is described at https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access.

Results

A total of 290 epithelial ovarian cancer cases were diagnosed in the NHS/NHSII after blood collection [median time between blood draw and diagnosis 10.9 years (interquartile range, IQR, 5.3–15.7)]. Of the 180,640 women in the UK Biobank with lipid measurements at baseline, 551 developed invasive ovarian cancer during a median 6.8 (IQR, 6.1–7.4) years of follow-up (**Table 1**). On average, cases in NHS/NHSII were 66.2 (range, 36–87) years old at diagnosis and in UK Biobank were 63.1 (range, 40–77) years old at diagnosis.

Total cholesterol and LDL-C were highly correlated (rho = 0.92 in plasma, rho = 0.95 in serum; Supplementary Table S1). Total cholesterol was modestly correlated with HDL-C (rho = 0.18 in plasma, rho = 0.29 in serum) and triglycerides (rho = 0.47 in plasma, rho = 0.29 in serum), as was LDL-C with triglycerides (rho = 0.37 in plasma, rho = 0.31 in serum). No correlation was noted between HDL-C and LDL-C (rho = −0.09 in plasma, rho = 0.03 in serum) while there was an inverse correlation between HDL-C and triglycerides (rho = −0.37 in plasma, rho = −0.41 in serum). The measured lipids were not highly correlated with previously measured CRP levels (rho < 0.20 for all comparisons). The measured triglycerides were correlated (median rho = 0.62, IQR = 0.45–0.72) with metabolomics measures of triglyceride species [saturated, monounsaturated, and polyunsaturated (up to 12 double bonds) with 42 to 60 Carbon atoms in the fatty acid chains] among controls (25).

There was a suggestion of a reduced ovarian cancer risk comparing the top to bottom quartile of total cholesterol [meta-analysis RR (95% CI): 0.81 (0.65–1.01); P_{trend} 0.06), with no heterogeneity across studies ($P_{heterogeneity}$ = 0.60; **Table 2**). Effect estimates for HDL-C and LDL-C were also less than 1; however, there were no clear patterns of reduced risk with increased levels for these lipids. There was no association between circulating triglycerides and ovarian cancer risk overall. HDL-C, LDL-C, and triglyceride–ovarian cancer associations were similar in models mutually adjusting for circulating lipid levels (**Table 2**) or CRP. Case/control counts for the quartile and clinical cut-off point analyses are reported in Supplementary Table S2.

In analyses evaluating associations among cases diagnosed more than 2 years after blood draw (n = 689 cases), women in the fourth versus first quartile of total cholesterol had a 24% lower risk of ovarian cancer (meta-analysis RR = 0.76; 95% CI, 0.59–0.97; **Table 3**, study specific results are reported in Supplementary Table S3). Patterns were similar for HDL-C and LDL-C, albeit not statistically significant. For these three markers (i.e., cholesterol, HDL-C, and LDL-C), the effect estimates were similar among cases diagnosed ≤2 years after blood draw (n = 152 cases; $P_{heterogeneity}$ > 0.09 across all strata), suggesting limited influence of preclinical cancer on circulating levels. In contrast, the association comparing extreme quartiles of triglycerides was heterogeneous across categories of time between blood draw and diagnosis [RR (95% CI), Q4 vs. Q1: ≤2 years between diagnosis and blood draw 0.51 (0.29–0.89) and >2 years 1.17 (0.90–1.52); $P_{heterogeneity}$ = 0.01], this difference was more extreme evaluating clinical cutpoints for triglycerides [RR (95% CI) >200 vs. ≤200 mg/dL: ≤2 years between diagnosis and blood draw 0.50 (0.27–0.93), n = 15 exposed vs. 137 unexposed cases; and >2 years 1.57 (1.03–2.42), n = 112 exposed vs. 577 unexposed; $P_{heterogeneity}$ = 0.003].

Associations across serous/non-serous subtype were remarkably consistent for total, HDL-C, and LDL-C. However, the association across serous/non-serous subtype for triglycerides was suggestive of an differential risk for triglycerides [RR (95% CI), Q4 vs. Q1: serous 1.26 (0.96–1.66); non-serous 0.92 (0.62–1.37); $P_{heterogeneity}$ = 0.207] (**Fig. 1**; Supplementary Table S4).

Although we did not observe statistically significant effect modification of the lipid-ovarian cancer associations by menopausal status/HT use and overweight/non-overweight BMI, some patterns emerged (**Fig. 1**; Supplementary Table S4). The inverse association of ovarian cancer with high cholesterol and triglycerides generally was stronger among postmenopausal women not using HT at blood draw or among women who were overweight or obese. For example, among postmenopausal women not using HT, we observed lower ovarian cancer risk with high levels of total cholesterol [RR (95% CI) Q4 vs. Q1: 0.58 (0.40–0.85); $P_{interaction}$ 0.08] and LDL-C [RR (95% CI) Q4 vs. Q1: 0.64 (0.44–0.93); $P_{interaction}$ 0.13] and elevated ovarian cancer risk with high levels of triglycerides [RR (95% CI) Q4 vs. Q1: 1.20 (0.81–1.78); $P_{interaction}$ 0.38]. We observed similar patterns of ovarian cancer risk among overweight/obese women [RR (95% CI) Q4 vs. Q1, total cholesterol: 0.69 (0.52–0.91); $P_{interaction}$ 0.09; HDL-C: 0.72 (0.53–0.98), $P_{interaction}$ 0.21; triglycerides: 1.31 (0.91–1.89); $P_{interaction}$ 0.33]. In contrast, associations were largely null when evaluating the

Table 1. Study population characteristics by ovarian cancer status, including data from a case–control study nested in the NHS and NHSII populations and a cohort study from the UK Biobank.

	NHS/NHSII (nested case–control study)				UK Biobank			
	Ovarian cancers ($n = 290$)		Controls ($n = 290$)		Ovarian cancers ($n = 551$)		Non-cases ($n = 180,089$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age at blood draw	55.6	7.9	55.7	7.5	59.0	7.4	56.1	8.0
Time between blood draw and diagnosis (years)	10.6	6.3			4.1	2.2		
High-sensitivity CRP	2.9	4.3	2.5	4.4	2.9	4.8	2.6	4.3
	n	%	*n*	%	*n*	%	*n*	%
BMI								
<25	177	61.0	170	58.6	201	36.5	72,518	40.3
25–29.9	67	23.1	92	31.7	215	39.0	66,299	36.8
30+	46	15.9	28	9.7	101	18.3	27,506	15.3
Unknown/missing	0		0		34	6.2	13,766	7.6
Cohort								
NHS	240	82.8	240	82.8				
NHSII	50	17.2	50	17.2				
UK Biobank					551	100.0	180,089	100.0
Time of blood draw								
2am–7am	30	10.3	27	9.3	0	0.0	0	0.0
8am–1pm	228	78.6	242	83.5	245	44.5	82,456	45.8
2pm–1am	32	11.0	21	7.2	306	55.5	97,633	54.2
Fasting status								
Yes	181	62.4	195	67.2	16	2.9	5,726	3.2
No	109	37.6	95	32.8	535	97.1	174,363	96.8
Menopausal status/HT usage								
Premenopausal	91	31.4	91	31.4	85	15.4	49,406	27.4
Postmenopausal/no HT	81	27.9	86	29.7	221	40.1	62,925	34.9
Postmenopausal/HT	79	27.2	73	25.2	224	40.7	58,262	32.4
Unknown/missing	39	13.5	40	13.8	21	3.8	9,496	5.3
Duration of oral contraceptive (OC) use								
Never	137	47.2	133	45.9	22	4.0	5,939	3.3
<1 yr	34	11.7	32	11.0	30	5.4	7,827	4.4
1–<5 yr	67	23.1	51	17.6	67	12.2	19,463	10.8
5+ yr	51	17.6	62	21.4	226	41.0	95,554	53.1
Unknown/missing	1	0.3	12	4.1	206	37.4	51,306	28.5
Parity								
0	31	10.7	17	5.9	123	22.3	33,575	18.6
1	17	5.9	11	3.8	87	15.8	23,796	13.2
2	95	32.8	85	29.3	219	39.8	80,327	44.6
3	78	26.9	88	30.3	91	16.5	31,881	17.7
4+	69	23.8	89	30.7	31	5.6	10,510	5.8
Tubal ligation								
No	245	84.5	234	80.7	516	93.7	165,502	91.9
Yes	45	15.5	56	19.3	35	6.4	14,587	8.1
Hysterectomy								
No	209	72.1	215	74.1	471	85.5	158,601	88.1
Yes	81	27.9	75	25.9	80	14.5	21,488	11.9
Unilateral oophorectomy								
No	275	94.8	275	94.8	547	99.3	178,230	99.0
Yes	15	5.2	15	5.2	4	0.7	1,859	1.0

Abbreviation: yr, years.

lipid-ovarian cancer associations among premenopausal women, postmenopausal women using HT at blood draw, and women with BMI less than 25 kg/m^2.

Discussion

Data from two large cohorts in the United States and United Kingdom suggest that lipid levels may be modestly association with ovarian cancer risk, although the direction of association varied by biomarker. Total cholesterol levels were inversely associated with overall ovarian cancer risk, associations were stronger for postmenopausal women not using HT and women with overweight/obese BMI. Consistent inverse associations with total cholesterol were observed for both serous and non-serous tumors. The pattern of association observed for total cholesterol was also observed when evaluating HDL-C and LDL-C; however, most associations did not reach statistical significance and require further exploration. Conversely, there was a suggestion of a possible increased ovarian cancer risk with high versus

Table 2. Circulating lipid measurements and ovarian cancer risk by study (NHS, NHSII, and UK Biobank) and summarized via meta-analysis.

	Fully adjusted models (model 1)							Mutually and fully adjusted models (model 2)						
	UKB		NHS/NHSII		Meta-analysis			UKB		NHS/NHSII		Meta-analysis		
	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	P_{het}	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	P_{het}
Cholesterol (mg/dL)														
Q1: <196	1	(ref)	1	(ref)	1	(ref)								
Q2: 196–222	0.76	(0.59–0.98)	1.06	(0.65–1.73)	0.82	(0.65–1.02)	0.24							
Q3: 223–255	0.91	(0.72–1.14)	1.34	(0.82–2.17)	0.98	(0.79–1.20)	0.16							
Q4: >255	0.83	(0.65–1.07)	0.72	(0.43–1.21)	0.81	(0.65–1.01)	0.60							
P_{trend}		0.08		0.41		0.06								
>240 vs. ≤240	0.98	(0.82–1.17)	0.79	(0.54–1.16)	0.94	(0.80–1.11)	0.31							
HDL cholesterol (mg/dL)														
Q1: <52	1	(ref)	1	(ref)	1	(ref)		1	(ref)	1	(ref)			
Q2: 52–59	1.01	(0.79–1.28)	0.91	(0.53–1.52)	0.99	(0.80–1.23)	0.74	1.01	(0.79–1.28)	0.98	(0.58–1.66)	1.00	(0.80–1.25)	0.94
Q3: 60–69	0.88	(0.69–1.12)	1.00	(0.62–1.62)	0.90	(0.73–1.12)	0.64	0.88	(0.69–1.13)	1.10	(0.66–1.81)	0.92	(0.74–1.15)	0.44
Q4: >69	0.83	(0.65–1.05)	1.06	(0.64–1.74)	0.87	(0.70–1.07)	0.39	0.83	(0.64–1.08)	1.19	(0.70–2.02)	0.89	(0.71–1.12)	0.24
P_{trend}		0.10		0.71		0.19			0.08		0.30		0.26	
<40 vs. ≥40	1.25	(0.85–1.84)	1.07	(0.36–3.23)	1.23	(0.85–1.77)	0.80	1.31	(0.89–1.95)	0.77	(0.26–2.31)	1.24	(0.85–1.79)	0.37
LDL cholesterol (mg/dL)														
Q1: <109	1	(ref)	1	(ref)	1	(ref)		1	(ref)	1	(ref)			
Q2: 109–131	0.92	(0.71–1.20)	0.96	(0.59–1.55)	0.93	(0.74–1.17)	0.88	0.93	(0.71–1.20)	0.91	(0.56–1.49)	0.92	(0.73–1.16)	0.96
Q3: 132–156	0.87	(0.67–1.12)	1.06	(0.65–1.72)	0.91	(0.72–1.14)	0.48	0.87	(0.68–1.13)	1.00	(0.61–1.63)	0.90	(0.72–1.13)	0.64
Q4: >156	0.93	(0.72–1.20)	0.71	(0.42–1.19)	0.89	(0.70–1.11)	0.36	0.94	(0.72–1.23)	0.62	(0.36–1.06)	0.87	(0.68–1.10)	0.17
P_{trend}		0.23		0.29		0.12			0.40		0.10		0.14	
>160 vs. ≤160	0.94	(0.78–1.14)	0.61	(0.39–0.95)	0.88	(0.74–1.05)	0.08	0.97	(0.80–1.18)	0.59	(0.37–0.92)	0.90	(0.75–1.07)	0.04
Triglycerides (mg/dL)														
Q1: <79	1	(ref)	1	(ref)	1	(ref)		1	(ref)	1	(ref)			
Q2: 79–108	1.04	(0.79–1.36)	1.03	(0.62–1.70)	1.04	(0.82–1.31)	0.97	1.03	(0.79–1.35)	1.12	(0.67–1.87)	1.05	(0.83–1.34)	0.79
Q3: 109–158	1.08	(0.83–1.41)	1.47	(0.88–2.43)	1.16	(0.92–1.46)	0.30	1.05	(0.80–1.39)	1.78	(1.03–3.07)	1.17	(0.92–1.50)	0.09
Q4: >158	1.08	(0.82–1.41)	1.30	(0.76–2.22)	1.12	(0.88–1.43)	0.55	1.01	(0.74–1.37)	1.72	(0.93–3.19)	1.12	(0.85–1.48)	0.13
P_{trend}		0.67		0.22		0.87			0.45		0.06		0.85	
>200 vs. ≤200	0.84	(0.66–1.07)	1.19	(0.71–1.99)	0.89	(0.72–1.11)	0.23	0.82	(0.64–1.05)	1.42	(0.83–2.43)	0.90	(0.72–1.13)	0.07

Note: Model 1: Adjusted for age (continuous), blood draw time (2am–7am, 8am–1pm, 2pm–1am), menopausal status/HT use (premenopausal, postmenopausal/no HT use, postmenopausal/HT use, unknown), and fasting status (no, yes) and OC use (never, <1, 1–<5, 5+ years), parity (nulliparous, 1, 2, 3, 4+ children), BMI (<25, 25–<30, 30–<35, ≥35), tubal ligation (no, yes), hysterectomy (no, yes), and unilateral oophorectomy (no, yes); NHS, additionally adjusted for cohort (NHS, NHSII). Model 2: Adjusted for variables in Model 1 plus the other circulating measures (e.g., LDL model was additionally adjusted for HDL and triglyceride levels). Abbreviation: P_{het}, *P* value testing heterogeneity of association across study. Case–control counts by lipid category provided in Supplementary Table S2.

low triglyceride levels, that was apparent when evaluating associations with ovarian cancers diagnosed more than 2 years after blood draw.

Our finding of modest reductions in risk across extreme quartiles of circulating cholesterol levels are in contrast to the mostly null associations observed in prior prospective studies (6–9) with effect estimates across extreme quantiles ranging from 1.07 to 2.17 for total cholesterol. Prior studies evaluating triglycerides have also varied, with effect estimates across extreme quantiles ranging from 0.93 to 1.36 (7–9). Inconsistent relationships between blood lipids and ovarian cancer risk in observational studies may be due to small sample sizes, varying timing/characteristics of blood collection, different assay methodology, and differential adjustment for confounding factors. In the current study, clinical assays were used to measure lipids in both populations and analyses were consistently adjusted for confounding across study. A prior study in the NHS/NHSII study populations reported an increased ovarian cancer risk with high levels of circulating triacylglycerols, as measured by a metabolomics platform. In overlapping controls from the two populations, triglycerides were correlated with triacylglycerols (25). The current suggestion of an increased risk of ovarian cancer with high relative to low levels of circulating triglycerides is consistent with this previous evaluation.

Mechanisms underlying the effects of decreased total cholesterol, LDL-C, and HDL-C on ovarian cancer risk are unclear as reports of the effects of cholesterol in the literature are conflicting. Lipids have multiple functions, such as cell growth, division, and signaling that are dysregulated with cancer (26). As such the timing of the measurement of lipids in assessing cancer risk in women may need to be considered. Importantly, in our study, the associations for cholesterol remained and for triglycerides became stronger in analyses limited to blood collected more than 2 years prior to diagnosis, suggesting that alterations in lipid levels due to subclinical disease could be masking an apparent association with ovarian cancer risk in prior studies. LDL-C has been shown to demonstrate cancer cell cytotoxicity and inhibit angiogenesis (27), a key oncogenic process, thus an inverse association is plausible. Endoplasmic reticulum (ER) stress may be another mechanism influencing cholesterol metabolism. Experimentally, ER stress dysregulates cholesterol metabolism, and cholesterol in the tumor microenvironment can induce ER stress and ultimately reducing antitumor immunity (28–32). T-cell activity has also been shown to vary by intracellular cholesterol levels (33–35). It is well established that cancer arises in the context of an *in vivo* tumor microenvironment which is both a cause and consequence of tumorigenesis. Cholesterol metabolism is one of many targets that may mediate anti-cancer effects

Table 3. Lipid–ovarian cancer associations stratified by time between blood draw and diagnosis, meta-analysis of data from prospective studies in the NHS, NHSII, and UK Biobank.

	Meta-analysis						
	≤2 years (n = 152 cases)			>2 years (n = 689 cases)			
	n cases	RR	(95% CI)	n cases	RR	(95% CI)	P_{het}
Cholesterol (mg/dL)							
Q1: <196	38	1	(ref)	175	1	(ref)	
Q2: 196–222	35	0.92	(0.57–1.48)	142	0.80	(0.63–1.03)	0.62
Q3: 223–255	43	0.89	(0.57–1.40)	214	0.94	(0.75–1.19)	0.84
Q4: >255	46	0.74	(0.46–1.19)	158	0.76	(0.59–0.97)	0.94
>240 vs. ≤240	55 vs. 97	0.62	(0.23–1.68)	257 vs. 432	0.93	(0.70–1.24)	0.76
HDL cholesterol (mg/dL)							
Q1: <52	44	1	(ref)	179	1	(ref)	
Q2: 52–59	22	0.67	(0.40–1.12)	154	1.09	(0.86–1.38)	0.09
Q3: 60–69	47	1.13	(0.73–1.73)	170	0.87	(0.68–1.10)	0.30
Q4: >69	39	0.88	(0.55–1.39)	186	0.90	(0.71–1.13)	0.92
<40 vs. ≥40	5 vs. 147	0.62	(0.23–1.68)	30 vs. 659	1.35	(0.62–2.93)	0.23
LDL cholesterol (mg/dL)							
Q1: <109	29	1	(ref)	147	1	(ref)	
Q2: 109–131	35	0.91	(0.54–1.51)	162	0.91	(0.70–1.17)	0.99
Q3: 132–156	48	1.05	(0.65–1.68)	182	0.85	(0.66–1.09)	0.45
Q4: >156	40	0.72	(0.44–1.18)	198	0.84	(0.65–1.08)	0.58
>160 vs. ≤160	36 vs. 116	0.78	(0.51–1.20)	168 vs. 521	0.84	(0.61–1.17)	0.78
Triglycerides (mg/dL)							
Q1: <79	28	1	(ref)	127	1	(ref)	
Q2: 79–108	38	1.00	(0.61–1.65)	154	1.01	(0.78–1.32)	0.96
Q3: 109–158	58	1.19	(0.74–1.90)	192	1.07	(0.82–1.39)	0.70
Q4: >158	28	0.51	(0.29–0.89)	216	1.17	(0.90–1.52)	0.01
>200 vs. ≤200	15 vs. 137	0.50	(0.27–0.93)	112 vs. 577	1.57	(1.03–2.42)	0.003

Note: Adjusted for age (continuous), blood draw time (2am–7am, 8am–1pm, 2pm–1am), menopausal status/HT use (premenopausal, postmenopausal/no HT use, postmenopausal/HT use, unknown), and fasting status (no, yes) and OC use (never, <1, 1-<5, 5+ years), parity (nulliparous, 1, 2, 3, 4+ children), BMI (<25, 25-<30, 30-<35, ≥35), tubal ligation (no, yes), hysterectomy (no, yes), and unilateral oophorectomy (no, yes); NHS—additionally adjusted for cohort (NHS, NHSII).
Abbreviation: P_{het}, P value testing heterogeneity of association across time between blood draw and diagnosis (≤2 and >2 years).

through the tumor microenvironment (36). Cholesterol is also thought to increase female cancer risk via increasing circulating estrogen levels, which may be particularly relevant in the low estrogen environment characteristic of postmenopausal women not using HT; however, we observed reductions in risk associated with increased circulating cholesterol within this group. Furthermore, estrogen levels appear to be primarily associated with risk of non-serous tumors (37) and we did not observe differential associations by this binary histology classification in our analysis, suggesting other potential pathways by which cholesterol could influence ovarian cancer development. The data on triglycerides and circulating hormone levels are limited, but suggests an inverse association with estrogens (38), which does not help to explain the increased ovarian cancer risk observed in the current study with high (relative to low) triglyceride levels.

Our study has several important strengths, including its large sample size and the inclusion of studies conducted in both the United States and United Kingdom. Additional strengths include the measurement of total cholesterol as well as the lipoprotein subfractions, HDL-C, LDL-C, and importantly triglycerides. Comprehensive

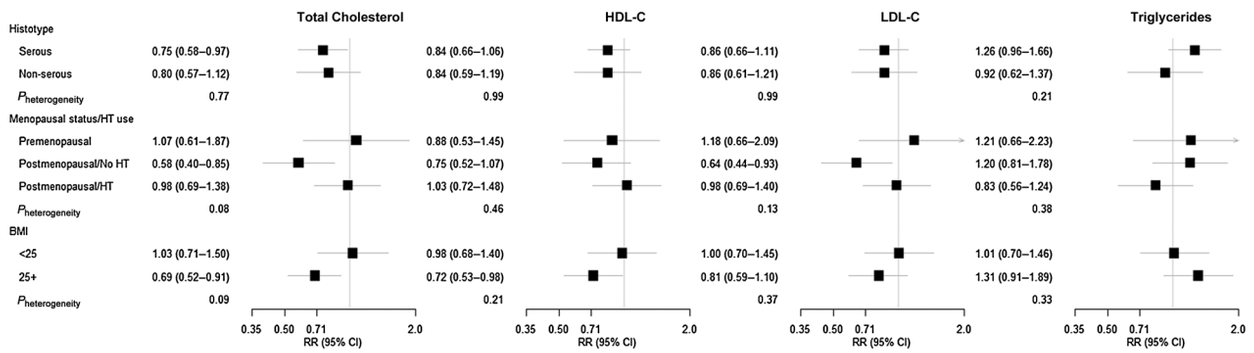

Figure 1.
Lipid–ovarian cancer associations (quartile 4 vs. quartile 1) by serous/non-serous subtype, menopausal status/HT use, and non-overweight/overweight BMI. Box represents RR, error bars 95% CIs.

information on potential confounding factors and equivalent adjustment across study populations are also notable strengths. Finally, given that we utilized data from prospective cohorts, we were able to assess the potential bias caused by preclinical disease by evaluating cases diagnosed at least 2 years after blood collection (23). However, generalizability of study is limited in that the summarized data are from predominantly non-Hispanic white study populations and should be explored more widely given that lipid profile components vary depending on race/ethnicity (39). Our study is limited in that we did not have a sample size sufficiently large enough to evaluate associations with rare ovarian cancer histotypes. We were unable to evaluate high-grade serous cancers because of missing information on tumor grade in the UK Biobank. We were also limited in our ability to evaluate our results in the context of lipid-lowering medications as this information was not uniformly available in the NHS/NHSII study population. Future analyses should consider both statin use as well as evaluating associations stratified by hypercholesterolemia.

This analysis of two large prospective studies suggests that circulating lipid levels are not strongly associated with ovarian cancer risk. The inverse association between total cholesterol and ovarian cancer incidence is not likely explained by undiagnosed/preclinical cancer. The positive association suggested between triglycerides and increased ovarian cancer risk warrants further evaluation, with a focus on factors that might modify this association (i.e., menopausal status and histotype).

Authors' Disclosures

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Authors' Contributions

B. Trabert: Data curation, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **C.A. Hathaway:** Data curation, formal analysis, investigation, writing–review and editing. **M.S. Rice:** Formal analysis, writing–review and editing. **E.B. Rimm:** Data curation, methodology, writing–review and editing. **P.M. Sluss:** Data curation, methodology, writing–review and editing. **K.L. Terry:** Methodology, writing–review and editing. **O.A. Zeleznik:** Methodology, writing–review and editing. **S.S. Tworoger:** Conceptualization, resources, data curation, supervision, investigation, methodology, writing–review and editing.

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