Mendelian randomization analyses suggest a role for cholesterol in the development of endometrial cancer

Pik Fang Kho[1, 2], Frederic Amant[3], Daniela Annibali[3], Katie Ashton[4-6], John Attia[4, 7], Paul L. Auer[8, 9], Matthias W. Beckmann[10], Amanda Black[11], Louise Brinton[11], Daniel D. Buchanan[12-15], Stephen J. Chanock[16], Chu Chen[17], Maxine M. Chen[18], Timothy H.T. Cheng[19], Linda S. Cook[20, 21], Marta Crous-Bous[18, 22], Kamila Czene[23], Immaculata De Vivo[18, 22], Joe Dennis[24], Thilo Dörk[25], Sean C. Dowdy[26], Alison M. Dunning[27], Matthias Dürst[28], Douglas F. Easton[24, 27], Arif B. Ekici[29], Peter A. Fasching[10, 30], Brooke L. Fridley[31], Christine M. Friedenreich[21], Montserrat García-Closas[16], Mia M. Gaudet[32], Graham G. Giles[13, 33, 34], Ellen L. Goode[35], Maggie Gorman[19], Christopher A. Haiman[36], Per Hall[23, 37], Susan E. Hankinson[22, 38], Alexander Hein[10], Peter Hillemanns[25], Shirley Hodgson[39], Erling Hoivik[40, 41], Elizabeth G. Holliday[4, 7], David J. Hunter[18, 42, 43], Angela Jones[19], Peter Kraft[18, 42], Camilla Krakstad[40, 41], Diether Lambrechts[44, 45], Loic Le Marchand[46], Xiaolin Liang[47], Annika Lindblom[48, 49], Jolanta Lissowska[50], Jirong Long[51], Lingeng Lu[52], Anthony M. Magliocco[53], Lynn Martin[54], Mark McEvoy[7], Roger L. Milne[13, 33, 34], Miriam Mints[55], Rami Nassir[56], Irene Orlow[47], Geoffrey Otton[57], Claire Palles[19], Loreall Pooler[36], Jennifer Prescott[22], Tony Proietto[57], Timothy R. Rebbeck[58, 59], Stefan P. Renner[60], Harvey A. Risch[52], Matthias Rübner[60], Ingo Runnebaum[28], Carlotta Sacerdote[61, 62], Gloria E. Sarto[63], Fredrick Schumacher[64], Rodney J. Scott[4, 6, 65], V. Wendy Setiawan[36], Mitul Shah[27], Xin Sheng[36], Xiao-Ou Shu[51], Melissa C. Southey[12, 33, 34], Emma Tham[48, 66], Ian Tomlinson[19, 54], Jone Trovik[40, 41], Constance Turman[18], Jonathan P. Tyrer[27], David Van Den Berg[36], Zhaoming Wang[11], Nicolas Wentzensen[11], Lucy Xia[36], Yong-Bing Xiang[67], Hannah P. Yang[11], Herbert Yu[46], Wei Zheng[51], Penelope M. Webb[68], Deborah J. Thompson[24], Amanda B. Spurdle[1], Dylan M. Glubb[1#], Tracy A. O'Mara[1#*]

[1] Department of Genetics and Computational Biology, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.
[2] School of Biomedical Science, Queensland University of Technology, Brisbane, Queensland, Australia.
[3] Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University Hospitals KU Leuven, University of Leuven, Leuven, Belgium.
[4] Hunter Medical Research Institute, John Hunter Hospital, Newcastle, New South Wales, Australia.
[5] Centre for Information Based Medicine, University of Newcastle, Callaghan, New South Wales, Australia.
[6] Discipline of Medical Genetics, School of Biomedical Sciences and Pharmacy, Faculty of Health, University of Newcastle, Callaghan, New South Wales, Australia.
[7] Centre for Clinical Epidemiology and Biostatistics, School of Medicine and Public Health, University of Newcastle, Callaghan, New South Wales, Australia.
[8] Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[9] Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI, USA.
[10] Department of Gynecology and Obstetrics, Comprehensive Cancer Center ER-EMN, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany.
[11] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA.
[12] Department of Clinical Pathology, The University of Melbourne, Melbourne, Victoria, Australia.
[13] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia.
[14] Genomic Medicine and Family Cancer Clinic, Royal Melbourne Hospital, Parkville, Victoria, Australia.
[15] University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria, Australia.
[16] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA.

[17] Epidemiology Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[18] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[19] Wellcome Trust Centre for Human Genetics and Oxford NIHR Biomedical Research Centre, University of Oxford, Oxford, UK.
[20] University of New Mexico Health Sciences Center, University of New Mexico, Albuquerque, NM, USA.
[21] Department of Cancer Epidemiology and Prevention Research, Alberta Health Services, Calgary, AB, Canada.
[22] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.
[23] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.
[24] Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.
[25] Gynaecology Research Unit, Hannover Medical School, Hannover, Germany.
[26] Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Mayo Clinic, Rochester, MN, USA.
[27] Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK.
[28] Department of Gynaecology, Jena University Hospital - Friedrich Schiller University, Jena, Germany.
[29] Institute of Human Genetics, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany.
[30] David Geffen School of Medicine, Department of Medicine Division of Hematology and Oncology, University of California at Los Angeles, Los Angeles, CA, USA.
[31] Department of Biostatistics, Kansas University Medical Center, Kansas City, KS, USA.
[32] Behavioral and Epidemiology Research Group, American Cancer Society, Atlanta, GA, USA.
[33] Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Victoria, Australia.
[34] Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia.
[35] Department of Health Science Research, Division of Epidemiology, Mayo Clinic, Rochester, MN, USA.
[36] Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.
[37] Department of Oncology, Södersjukhuset, Stockholm, Sweden.
[38] Department of Biostatistics & Epidemiology, University of Massachusetts, Amherst, Amherst, MA, USA.
[39] Department of Clinical Genetics, St George's, University of London, London, UK.
[40] Centre for Cancer Biomarkers CCBIO, Department of Clinical Science, University of Bergen, Bergen, Norway.
[41] Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway.
[42] Program in Genetic Epidemiology and Statistical Genetics, Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[43] Nuffield Department of Population Health, University of Oxford, Oxford, UK.
[44] VIB Center for Cancer Biology, Leuven, Belgium.
[45] Laboratory for Translational Genetics, Department of Human Genetics, University of Leuven, Leuven, Belgium.
[46] Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, USA.
[47] Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
[48] Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.
[49] Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

[50] Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Cancer Center, Oncology Institute, Warsaw, Poland.
[51] Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA.
[52] Chronic Disease Epidemiology, Yale School of Medicine, New Haven, CT, USA.
[53] Department of Anatomic Pathology, Moffitt Cancer Center & Research Institute, Tampa, FL, USA.
[54] Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK.
[55] Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden.
[56] Department of Biochemistry and Molecular Medicine, University of California Davis, Davis, CA, USA.
[57] School of Medicine and Public Health, University of Newcastle, Callaghan, New South Wales, Australia.
[58] Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[59] Dana-Farber Cancer Institute, Boston, MA, USA.
[60] Department of Gynaecology and Obstetrics, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany.
[61] Center for Cancer Prevention (CPO-Peimonte), Turin, Italy.
[62] Human Genetics Foundation (HuGeF), Turino, Italy.
[63] Department of Obstetrics and Gynecology, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA.
[64] Department of Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, OH, USA.
[65] Division of Molecular Medicine, Pathology North, John Hunter Hospital, Newcastle, New South Wales, Australia.
[66] Clinical Genetics, Karolinska Institutet, Stockholm, Sweden.
[67] State Key Laboratory of Oncogene and Related Genes & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.
[68] Population Health Department, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.

#These authors contributed equally to this work

Corresponding Author: Dr Tracy O'Mara, PhD, Molecular Cancer Epidemiology Group, QIMR Berghofer Medical Research Institute, 300 Herston Road, Brisbane QLD Australia 4006. Phone: +61 7 3362 0389. Email: Tracy.OMara@qimrberghofer.edu.au

Short title: The effects of blood lipid levels on endometrial cancer development

Keywords: Mendelian randomization, endometrial cancer risk, LDL cholesterol, HDL cholesterol, triglycerides

Article Category: Cancer Epidemiology

Funding: This work was supported by a National Health and Medical Research Council (NHMRC) Project Grant (APP1109286). PFK is supported by an Australian Government Research Training Program PhD Scholarship and QIMR Berghofer Postgraduate Top-Up Scholarship, TAO'M is supported by an NHMRC Early Career Fellowship (APP1111246), ABS is supported by an NHMRC Senior Research Fellowship (APP1061779).

Conflict of Interest Statement: The authors declare no potential conflicts of interest.

Novelty and Impact Statement: This is the first study to use Mendelian randomization analysis to explore the relationship between blood lipids and risk of endometrial cancer and its subtypes. Genetically predicted lower LDL cholesterol levels or higher HDL cholesterol levels were associated with increased non-endometrioid endometrial cancer risk. Further work is required to elucidate the biology underlying these associations. These results indicate that cholesterol levels could be considered risk factors for endometrial cancer, and studies are required to assess the clinical significance of this association.

Abbreviations:

LDL: low-density lipoprotein

HDL: high-density lipoprotein

BMI: body mass index

GWAS: genome-wide association study

mtCOJO: multi-trait-based conditional and joint analysis

GSMR: Generalised Summary-data based Mendelian Randomisation

HEIDI: Heterogeneity in Dependent Instruments

LD: linkage disequilibrium

OR: odds ratio

CI: confidence interval

Abstract

Blood lipids have been associated with the development of a range of cancers, including breast, lung and colorectal cancer. For endometrial cancer, observational studies have reported inconsistent associations between blood lipids and cancer risk. To reduce biases from unmeasured confounding, we performed a bidirectional, two-sample Mendelian randomization analysis to investigate the relationship between levels of three blood lipids (low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglycerides) and endometrial cancer risk. Genetic variants associated with each of these blood lipid levels ($P < 5\times10^{-8}$) were identified as instrumental variables, and assessed using genome-wide association study data from the Endometrial Cancer Association Consortium (12,906 cases and 108,979 controls) and the Global Lipids Genetic Consortium (n=188,578). Mendelian randomization analyses found genetically raised LDL cholesterol levels to be associated with lower risks of endometrial cancer of all histologies combined, and of endometrioid and non-endometrioid subtypes. Conversely, higher genetically predicted HDL cholesterol levels were associated with increased risk of non-endometrioid endometrial cancer. After accounting for the potential confounding role of obesity (as measured by body mass index), the association between genetically predicted increased LDL cholesterol levels and lower endometrial cancer risk remained significant, especially for non-endometrioid endometrial cancer. There was no evidence to support a role for triglycerides in endometrial cancer development. Our study supports a role for LDL and HDL cholesterol in the development of non-endometrioid endometrial cancer. Further studies are required to understand the mechanisms underlying these findings.

Introduction

Endometrial cancer primarily affects postmenopausal women and approximately 382,000 cases were diagnosed in 2018[1]. Obesity is the strongest risk factor for endometrial cancer, with up to ~60% increased risk per 5 kg/m^2 higher body mass index (BMI)[2]. However, the mechanism(s) by which higher BMI predisposes to endometrial cancer are not well understood. Adipose tissue is an important site for the synthesis of estrogen (another endometrial cancer risk factor), especially after menopause, via the conversion of androgens to estrogens by aromatase[3]. BMI also has a complex relationship with blood lipid levels, with Mendelian randomization analyses finding bidirectional associations between levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides and BMI[4]. Moreover, cholesterol has been suggested to play a role in cancer development by inducing chronic inflammation[5-7].

Blood lipid levels have been suggested to contribute to pathogenesis of endometrial cancer. As hypertriglyceridemia and hyper-LDL cholesterolemia are common in endometrial cancer survivors[8], case-control studies assessing changes in blood lipid levels at/after endometrial cancer diagnosis are susceptible to reverse causation bias[9-11]. Observational studies conducted to examine the association between pre-diagnostic blood lipid levels and endometrial cancer risk[12-18] reported significant positive associations from only three studies assessing blood triglycerides level and endometrial cancer risk[13,14,18]. Inconsistent findings from observational studies could be due to small study populations[12,15] and a lack of adjustment for obesity[13,17]. Further, the use of non-fasting blood lipid levels in observational studies could also contribute to the variation in published findings[12-14,16-18]. Several studies have assessed the association of blood lipids with endometrial cancer by subtype[10,14,16,18], but only one has assessed the pre-diagnostic blood lipid levels. This study reported increased triglycerides levels to be associated with the risk of both type 1 and 2 endometrial cancers[18]. However, this study did not adjust for obesity, and used non-fasting blood lipid levels. As

obesity and blood lipid levels are interrelated[4], it has been difficult for observational studies to disentangle the effects of blood lipid levels on endometrial cancer risk. Thus, the relationship between blood lipids and endometrial cancer remains unclear from the existing evidence.

Mendelian randomization is an instrumental variable analysis that assesses the effects of exposures using genetic predictors as instrumental variables[19]. Mendelian randomization uses the principle that the alleles of genetic variants which predict higher levels of an exposure of interest are naturally randomized to individuals at meiosis, a process somewhat comparable to the random assignment of participants to an exposure in a randomized controlled trial. Thus, associations between genetic variants and the outcome (and hence between the exposure and the outcome) will not be vulnerable to reverse causation because disease develops after meiosis. Provided that the selected genetic variants are associated with the outcome only via their effects on the exposure of interest (i.e. not via pleiotropic effects on other traits which could independently alter disease risk), effect estimates generated by Mendelian randomization analyses should also be less vulnerable to the influence of confounders[19].

In the current study, we employed a two-sample Mendelian randomization framework to assess the relationships between levels of three blood lipids (LDL and HDL cholesterol, and triglycerides) and the risk of endometrial cancer using genome-wide association study (GWAS) data from the Endometrial Cancer Association Consortium (ECAC) and Global Lipids Genetic Consortium.

Materials and Methods

GWAS datasets

In this study, we assessed three major blood lipids: LDL and HDL cholesterol, and triglycerides. Summary statistics from GWAS for the three blood lipids in 188,577 individuals of predominantly European ancestry were obtained from the Global Lipid Genetics Consortium[20]

(http://csg.sph.umich.edu/willer/public/lipids2013/). For each genetic variant, the association estimates were expressed in standard deviation (SD) per copy of the effect allele.

Endometrial cancer risk estimates were obtained from the largest published meta-GWAS to date, conducted by ECAC in 12,906 endometrial cancer cases and 108,979 controls, all of European ancestry[21]. In a secondary analysis, we investigated relationships between the three blood lipids and endometrial cancer subtypes using ECAC meta-GWAS results restricted to cases with either endometrioid histology (8,758 cases), or non-endometrioid histology (1,230 cases)[21]. The association estimates were expressed in log(OR) per copy of the effect allele.

Instrumental variable selection

Independent, genome-wide significant genetic variants ($r^2 < 0.05$, $P < 5 \times 10^{-8}$) that were associated with each type of blood lipid were chosen as instrumental variables. Genetic variants with ambiguous strand codification (A/T or C/G) and minor allele frequency more than 0.42 were removed. We compared the allele frequencies between datasets of Global Lipids Genetics Consortium and a subset of ECAC (up to 25,902 cases and controls), and genetic variants with a large allele frequency difference (> 0.2) were also excluded.

Bidirectional Mendelian randomization analysis

We employed bidirectional Generalised Summary-data based Mendelian Randomisation (GSMR) analysis[22] to explore the relationship between the three blood lipids and endometrial cancer. As Mendelian randomization estimates may be confounded by including pleiotropic variants, we implemented the built-in Heterogeneity in Dependent Instruments (HEIDI) outlier test[22] with a P-value threshold of 0.01 to detect and filter heterogeneous variants that are likely pleiotropic. Remaining variants not excluded by HEIDI outlier test were used as non-pleiotropic instrumental variables.

Results with a Bonferroni-adjusted $P < 0.05/3 = 0.017$, correcting for the three blood lipid traits tested, were considered statistically significant. When blood lipid levels were treated as the exposure trait, the resulting effect estimates were expressed as odds ratios (OR) and 95% confidence intervals (CI) for endometrial cancer risk per SD increment in genetically predicted blood lipid level. When endometrial cancer risk was treated as the exposure trait, the resulting estimates represent the SD change for blood lipid level per SD increase in the genetic liability to endometrial cancer. Analyses were performed using default settings in the GSMR extension in GCTA (version 1.92)[22], using GWAS data from a subset of ECAC participants (up to 25,092 individuals) to calculate linkage disequilibrium (LD) between variants. For comparison we also performed inverse variance weighted (IVW) and MR-Egger regression Mendelian randomization analyses using MR-Base[23].

Conditional Mendelian randomization Analysis

Since obesity could affect associations between blood lipid levels and endometrial cancer[4], we additionally performed conditional Mendelian randomization analysis. GWAS summary statistics for the lipid of interest were conditioned for the effect of BMI using results from the largest GWAS of BMI to date[24]. Conditional analyses were performed using multi-trait-based conditional and joint analysis (mtCOJO) in the GCTA software package (version 1.92)[22] and adjusted estimates were then reanalysed by GSMR.

Results

After removal of potential pleiotropic variants, 145 LDL cholesterol, 171 HDL cholesterol and 101 triglyceride independent genome-wide significant variants were considered as instrumental variables (**Supplementary Table 1-3**). These instrumental variables were used by GSMR to estimate the effect of blood lipids on endometrial cancer risk of all histologies combined (results presented in **Table 1** and **Figure 1**). GSMR analysis indicated that genetically raised LDL cholesterol was associated with reduced risk of all endometrial cancer histologies combined (OR

per SD increase in LDL cholesterol level = 0.87; 95% CI = 0.83-0.92; $P = 2.73 \times 10^{-6}$). Consistent with the divergent roles of LDL and HDL cholesterol[25], GSMR analysis provided evidence that increased HDL cholesterol was associated with increased risk of all endometrial cancer histologies combined (OR 1.08; 95% CI = 1.01-1.15; P = 0.017). Secondary analysis assessing the relationships between blood lipid levels and endometrial cancer subtypes found genetically predicted higher LDL cholesterol level was associated with lower risk of both endometrioid and non-endometrioid endometrial cancer. Conversely, genetically predicted higher HDL cholesterol was only associated with higher risk of non-endometrioid endometrial cancer. No significant effects were observed for triglycerides on endometrial cancer overall, or its subtypes (**Table 1**). Bidirectional GSMR analysis provided evidence for a unidirectional association e.g. genetically elevated LDL cholesterol level may affect endometrial cancer risk, while genetic liability to endometrial cancer does not appear to affect LDL cholesterol levels (**Table 2**).

To reduce the influence of obesity on the associations between blood lipid levels and endometrial cancer risk, we performed Mendelian randomization analysis conditioning on BMI. Results are presented in **Table 3** and **Supplementary Figure 1**. After controlling for the influence of BMI, the association between genetically predicted LDL cholesterol levels and risk of all histologies combined and non-endometrioid endometrial cancer remained; whereas, the effect of LDL cholesterol level on endometrioid endometrial cancer risk was attenuated and no longer significant (OR 0.94, 95% CI 0.88-1.01; P = 0.12). Conditioning on BMI had minimal impact on the risk estimates for HDL and endometrial cancer, but associations no longer passed the Bonferroni-correction threshold, reflecting the decreased power for these analyses.

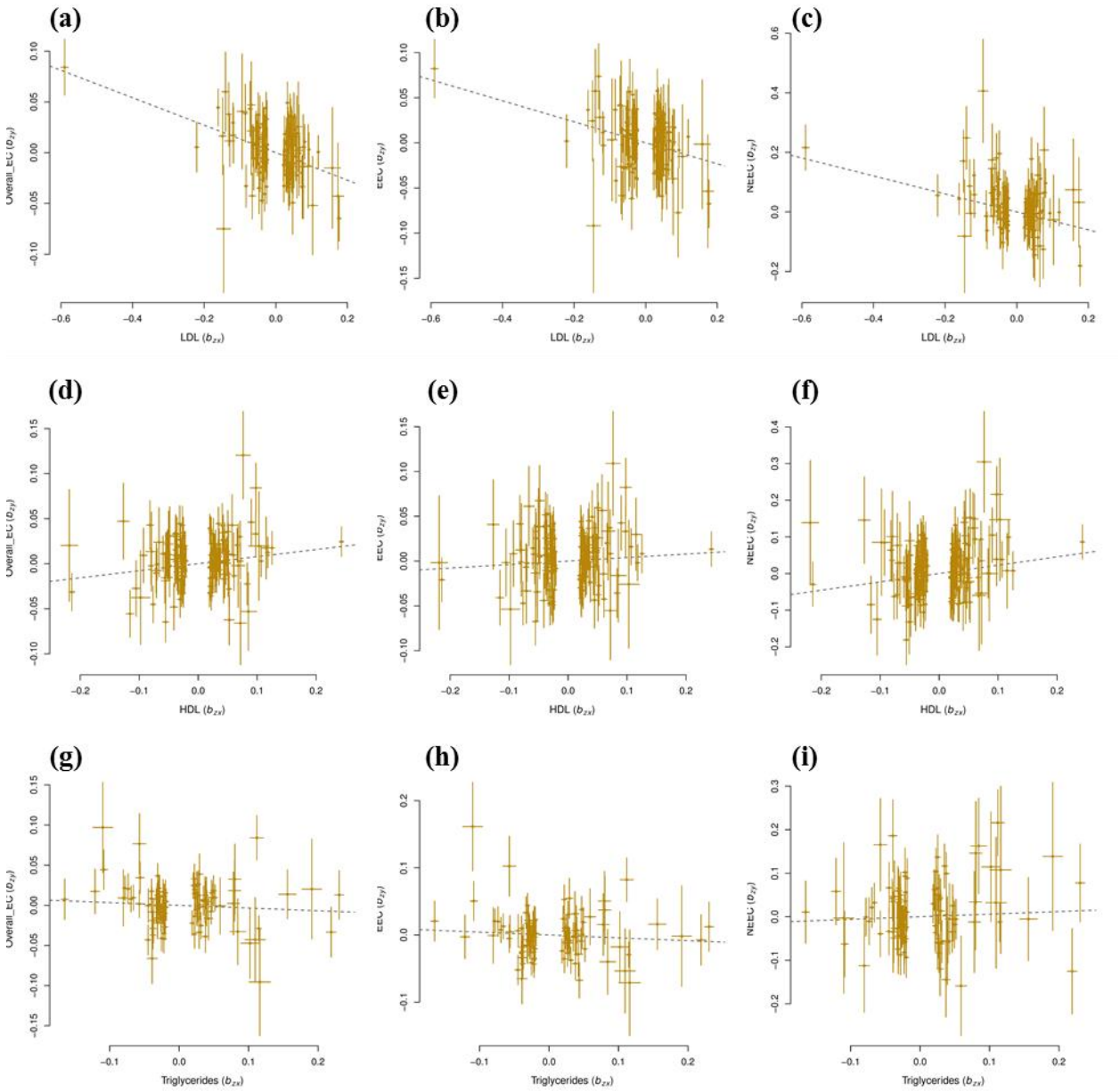


Figure 1. Effects of blood lipid trait instrumental variables on endometrial cancer risk. Figure showed the GSMR results for (a) LDL-endometrial cancer (all histologies combined), (b) LDL-endometrioid histology, (c) LDL-non-endometrioid histologies, (d) HDL-endometrial cancer (all histologies combined), (e) HDL-endometrioid histology, (f) HDL-non-endometrioid histology, (g) Triglycerides-endometrial cancer (all histologies combined), (h) Triglycerides-endometrioid histology, (i) Triglycerides-non-endometrioid histologies. The effect of each genetic variant for lipid of interest is plotted against the effect for the same variant on endometrial cancer risk. Dashed lines represent GSMR estimates.

Results from IVW and MR-Egger analyses were consistent with our GSMR results (**Supplementary Table 4**). None of the MR-Egger intercepts were significantly different from zero (P>0.05), suggesting pleiotropy did not bias the IVW results (**Supplementary Table 4**).

Discussion

To our knowledge, this is the first Mendelian randomization study to assess the effects of blood lipid levels on endometrial cancer risk. While genetically increased LDL cholesterol had a protective effect on endometrial cancer, especially non-endometrioid endometrial cancer, results suggest that genetically increased HDL cholesterol may have an adverse effect on non-endometrioid endometrial cancer risk. The opposing findings for LDL and HDL cholesterol are consistent with their opposing roles. For example, LDL can deliver cholesterol to peripheral tissues, whereas HDL removes cholesterol from these tissues and transports it to the liver[25]. We found no evidence of a causal link between triglycerides and endometrial cancer, in contrast to three observational studies that have reported positive associations[13,14,18]. However, as previously noted, none of these studies assessed fasting blood triglycerides and one did not control for the effect of obesity[13].

Mendelian randomization analysis has previously illustrated the complex interrelationship between BMI and blood lipid levels[4]. We therefore performed conditional Mendelian randomization analysis to investigate the influence of BMI on associations between LDL/HDL cholesterol and endometrial cancer risk. Comparison of the LDL/HDL cholesterol association estimates, before and after adjusting for BMI, did not support a role for BMI in the associations with endometrial cancer of non endometrioid and combined histologies. In contrast, the LDL cholesterol association with endometrioid endometrial cancer was weaker with wider confidence intervals after including BMI as covariate. While a modest protective

effect of LDL cholesterol for the endometrioid subtype of endometrial cancer cannot be excluded, this finding indicates that LDL cholesterol is likely to lie in the same causal pathway as obesity, a hypothesis consistent with results from previous genetic studies. Indeed, somewhat surprisingly, Mendelian randomization analysis has shown increasing LDL cholesterol is associated with reduced BMI[4] and also that increasing BMI is associated with increasing endometrioid endometrial cancer risk[26]. Thus, we hypothesise that obesity is likely to be the mediator of the effect of LDL cholesterol on endometrioid endometrial cancer risk (i.e. ↑LDL → ↓BMI → ↓Endometrioid Endometrial Cancer risk) (**Figure 2**). Moreover, as obesity is a stronger risk factor for endometrioid than for non-endometrioid endometrial cancer[21], it is perhaps not surprising that after adjusting for BMI we only observed an attenuation of the effect of LDL cholesterol on endometrioid endometrial cancer risk.

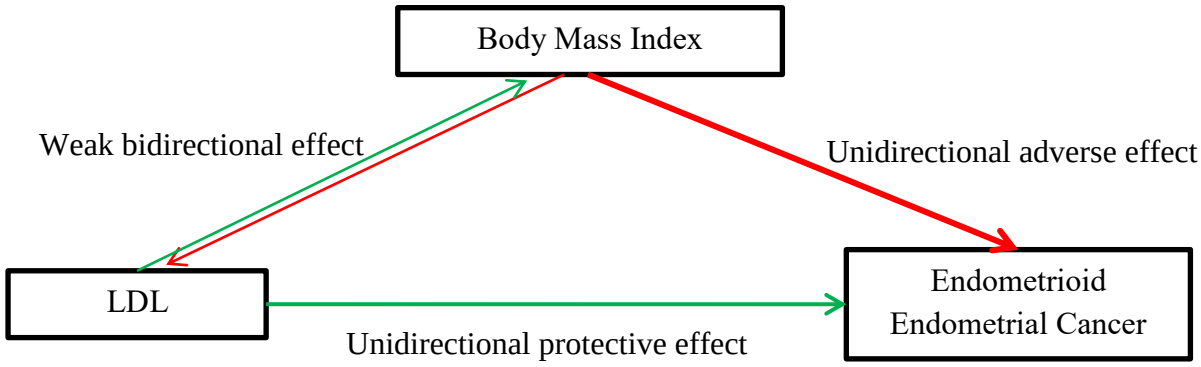


Figure 2. Relationships between LDL cholesterol, BMI and endometrioid endometrial cancer identified by previous[4,26] and current Mendelian randomization analysis. Red lines represent adverse effects, and green lines represent protective effects.

It is intriguing that our results indicated that, independent of obesity, decreased LDL cholesterol level is inversely associated with risk of non-endometrioid endometrial cancer. While both endometrioid and non-endometrioid endometrial cancer share many other risk factors[27], recent Mendelian randomization analyses have found that obesity and age at menarche are risk factors of endometrioid endometrial cancer only[21].Given the rare nature of

non-endometrioid histologies (~10% of all endometrial cancer cases), the tumorigenic mechanisms for these histological subtypes remain largely unknown[27,28]. Thus, further studies are required to explore how higher LDL cholesterol levels could protect against non-endometrioid endometrial cancer development.

As shown in **Table 1**, the association between HDL cholesterol and endometrial cancer appears to be largely driven by the non-endometrioid histological subtype. Despite not passing a Bonferroni statistical significance threshold, there was no substantial change in the association estimate before and after conditioning on BMI, suggesting HDL cholesterol may also affect non-endometrioid endometrial cancer risk independently of obesity. The wide confidence intervals suggest that future studies with more non-endometrioid endometrial cancer cases are required to further dissect any effect.

The conflicting findings regarding the relationships between blood lipids and endometrial cancer risk in observational studies may be due to small sample sizes, varying timing of blood collection (e.g. fasting or non-fasting, and pre- or post- endometrial cancer diagnosis), and varying control for confounding factors. Findings presented in the current study, through the application of bidirectional Mendelian randomization which is less vulnerable to reverse causation and confounding, have helped to clarify the effects of blood lipids on endometrial cancer risk. Consistent with our findings, other Mendelian randomization studies have observed a positive association between HDL cholesterol and breast cancer risk[29,30], and an inverse association between LDL cholesterol and lung cancer risk[31]. Similarly, a time-to-event Mendelian randomization using data from five longitudinal cohort studies reported increased LDL cholesterol level to be associated with reduced cancer risk (all reported cancer types combined)[32].

The validity of Mendelian randomization analysis lies upon the satisfaction of the assumption that the effect of the instrumental variables on the outcome is only mediated through their influence on the measured exposure (i.e. no horizontal pleiotropy). The HEIDI outlier test was used to improve the robustness of Mendelian randomization analysis by removing variants which show evidence of horizontal pleiotropy. The two-sample Mendelian randomization framework allowed us to incorporate data from two very large independent GWAS datasets to bolster power and yield more precise association estimates. However, we were restricted to summary-level GWAS data, and thus, could not perform more refined analyses (e.g. stratification analysis by BMI).

This Mendelian randomization study provides evidence that increased LDL cholesterol and decreased HDL cholesterol, independent of obesity, may reduce the risk of endometrial cancer. This effect was particularly apparent for the non-endometrioid endometrial cancer subtype, which typically has a more aggressive phenotype and results in poorer prognosis. Although further work is required to elucidate the biological rationale underlying this association, these results suggest low LDL cholesterol levels and high HDL cholesterol levels should be considered as potential risk factors for endometrial cancer.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;**68**: 394-424.

2. Aune D, Navarro Rosenblatt DA, Chan DS, Vingeliene S, Abar L, Vieira AR, Greenwood DC, Bandera EV, Norat T. Anthropometric factors and endometrial cancer risk: a systematic review and dose-response meta-analysis of prospective studies. *Annals of oncology : official journal of the European Society for Medical Oncology* 2015;**26**: 1635-48.

3. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;**89**: 2548-56.

4. Yang XL, Cui ZZ, Zhang H, Wei XT, Feng GJ, Liu L, Liu YZ, Pei YF, Zhang L. Causal link between lipid profile and bone mineral density: A Mendelian randomization study. *Bone* 2019;**127**: 37-43.

5. Bakiri L, Hamacher R, Grana O, Guio-Carrion A, Campos-Olivas R, Martinez L, Dienes HP, Thomsen MK, Hasenfuss SC, Wagner EF. Liver carcinogenesis by FOS-dependent inflammation and cholesterol dysregulation. *The Journal of experimental medicine* 2017;**214**: 1387-409.

6. Rossin D, Calfapietra S, Sottero B, Poli G, Biasi F. HNE and cholesterol oxidation products in colorectal inflammation and carcinogenesis. *Free radical biology & medicine* 2017;**111**: 186-95.

7. Du Q, Wang Q, Fan H, Wang J, Liu X, Wang H, Wang Y, Hu R. Dietary cholesterol promotes AOM-induced colorectal cancer through activating the NLRP3 inflammasome. *Biochemical pharmacology* 2016;**105**: 42-54.

8. Hirasawa A, Makita K, Akahane T, Yokota M, Yamagami W, Banno K, Susumu N, Aoki D. Hypertriglyceridemia is frequent in endometrial cancer survivors. *Japanese journal of clinical oncology* 2013;**43**: 1087-92.

9. Swanson CA, Potischman N, Barrett RJ, Berman ML, Mortel R, Twiggs LB, Wilbanks GD, Hoover RN, Brinton LA. Endometrial cancer risk in relation to serum lipids and lipoprotein levels. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1994;**3**: 575-81.

10. Zhang Y, Liu Z, Yu X, Zhang X, Lu S, Chen X, Lu B. The association between metabolic abnormality and endometrial cancer: a large case-control study in China. *Gynecologic oncology* 2010;**117**: 41-6.

11. Friedenreich CM, Biel RK, Lau DC, Csizmadi I, Courneya KS, Magliocco AM, Yasui Y, Cook LS. Case-control study of the metabolic syndrome and metabolic risk factors for endometrial cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011;**20**: 2384-95.

12. Lindemann K, Vatten LJ, Ellstrom-Engh M, Eskild A. Serum lipids and endometrial cancer risk: results from the HUNT-II study. *International journal of cancer* 2009;**124**: 2938-41.

13. Seth D, Garmo H, Wigertz A, Holmberg L, Hammar N, Jungner I, Lambe M, Walldius G, Van Hemelrijck M. Lipid profiles and the risk of endometrial cancer in the Swedish AMORIS study. *International journal of molecular epidemiology and genetics* 2012;**3**: 122-33.

14. Bjorge T, Stocks T, Lukanova A, Tretli S, Selmer R, Manjer J, Rapp K, Ulmer H, Almquist M, Concin H, Hallmans G, Jonsson H, et al. Metabolic syndrome and endometrial carcinoma. *American journal of epidemiology* 2010;**171**: 892-902.

15. Kabat GC, Kim MY, Chlebowski RT, Vitolins MZ, Wassertheil-Smoller S, Rohan TE. Serum lipids and risk of obesity-related cancers in postmenopausal women. *Cancer causes & control : CCC* 2018;**29**: 13-24.

16. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Tjonneland A, Olsen A, Overvad K, Jakobsen MU, Chajes V, Clavel-Chapelon F, Boutron-Ruault MC, et al. Metabolic syndrome, plasma lipid, lipoprotein and glucose levels, and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocrine-related cancer* 2007;**14**: 755-67.

17. Fortner RT, Husing A, Kuhn T, Konar M, Overvad K, Tjonneland A, Hansen L, Boutron-Ruault MC, Severi G, Fournier A, Boeing H, Trichopoulou A, et al. Endometrial

cancer risk prediction including serum-based biomarkers: results from the EPIC cohort. *International journal of cancer* 2017;**140**: 1317-23.

18. Trabert B, Wentzensen N, Felix AS, Yang HP, Sherman ME, Brinton LA. Metabolic syndrome and risk of endometrial cancer in the united states: a study in the SEER-medicare linked database. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2015;**24**: 261-7.

19. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. *JAMA* 2017;**318**: 1925-6.

20. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, et al. Discovery and refinement of loci associated with lipid levels. *Nature genetics* 2013;**45**: 1274-83.

21. O'Mara TA, Glubb DM, Amant F, Annibali D, Ashton K, Attia J, Auer PL, Beckmann MW, Black A, Bolla MK, Brauch H, Brenner H, et al. Identification of nine new susceptibility loci for endometrial cancer. *Nature communications* 2018;**9**: 3166.

22. Zhu Z, Zheng Z, Zhang F, Wu Y, Trzaskowski M, Maier R, Robinson MR, McGrath JJ, Visscher PM, Wray NR, Yang J. Causal associations between risk factors and common diseases inferred from GWAS summary data. *Nat Commun* 2018;**9**: 224.

23. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, Tan VY, Yarmolinsky J, et al. The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018;**7**.

24. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, Frayling TM, Hirschhorn J, Yang J, Visscher PM, Consortium G. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Human molecular genetics* 2018.

25. Feingold KR, Grunfeld C. Introduction to Lipids and Lipoproteins. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, et al. *Endotext*ed. South Dartmouth (MA), 2000.

26. Painter JN, O'Mara TA, Marquart L, Webb PM, Attia J, Medland SE, Cheng T, Dennis J, Holliday EG, McEvoy M, Scott RJ, Ahmed S, et al. Genetic Risk Score Mendelian Randomization Shows that Obesity Measured as Body Mass Index, but not Waist:Hip Ratio, Is Causal for Endometrial Cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2016;**25**: 1503-10.

27. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, Wolk A, Wentzensen N, Weiss NS, Webb PM, van den Brandt PA, van de Vijver K, et al. Type I and II endometrial cancers: have they different risk factors? *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013;**31**: 2607-18.

28. Yang HP, Wentzensen N, Trabert B, Gierach GL, Felix AS, Gunter MJ, Hollenbeck A, Park Y, Sherman ME, Brinton LA. Endometrial cancer risk factors by 2 main histologic subtypes: the NIH-AARP Diet and Health Study. *American journal of epidemiology* 2013;**177**: 142-51.

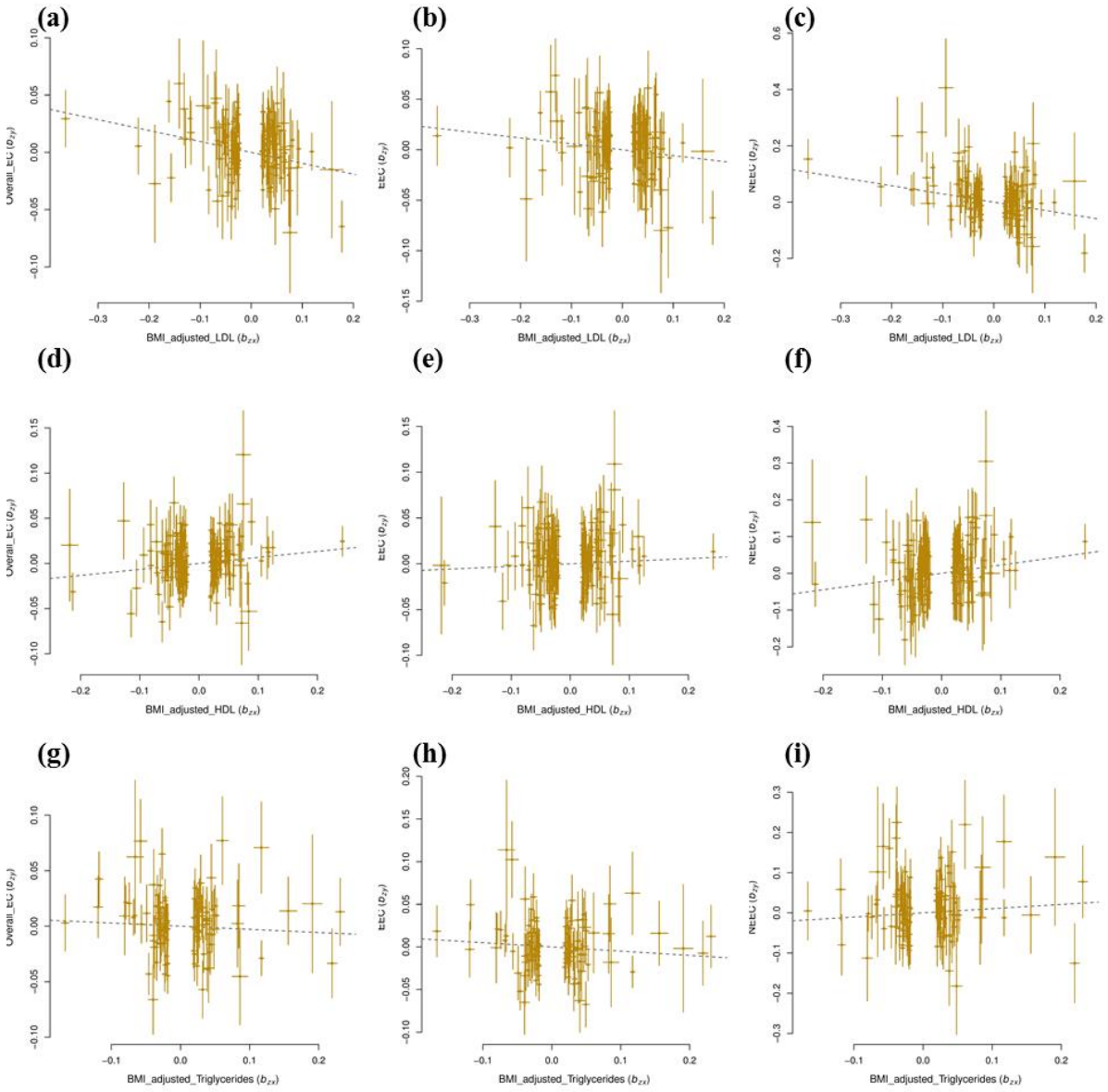
29. Nowak C, Arnlov J. A Mendelian randomization study of the effects of blood lipids on breast cancer risk. *Nature communications* 2018;**9**: 3957.

30. Johnson KE, Siewert KM, Klarin D, Damrauer SM, , Chang K-M, Tsao PS, Assimes TL, Maxwell KN, Voight BF. Assessing a causal relationship between circulating lipids and breast cancer risk: Mendelian randomization study. *bioRxiv* 2019.

31. Carreras-Torres R, Johansson M, Haycock PC, Wade KH, Relton CL, Martin RM, Davey Smith G, Albanes D, Aldrich MC, Andrew A, Arnold SM, Bickeboller H, et al.

Obesity, metabolic factors and risk of different histological types of lung cancer: A Mendelian randomization study. *PloS one* 2017;**12**: e0177875.

32. He L, Culminskaya I, Loika Y, Arbeev KG, Bagley O, Duan M, Yashin AI, Kulminski AM. Causal effects of cardiovascular risk factors on onset of major age-related diseases: A time-to-event Mendelian randomization study. *Experimental gerontology* 2018;**107**: 74-86.



Supplementary Figure 1. Effects of the blood lipid trait instrumental variables on endometrial cancer risk, after conditioning on BMI. Figure showed the GSMR results for (a) LDL-endometrial cancer (all histologies combined), (b) LDL-endometrioid histology, (c) LDL-non-endometrioid histologies, (d) HDL-endometrial cancer (all histologies combined), (e) HDL-endometrioid histologies, (f) HDL-non-endometrioid histologies, (g) Triglycerides-endometrial cancer (all histologies combined), (h) Triglycerides-endometrioid histologies and (i) Triglycerides-non-endometrioid histologies. The effect of each genetic variant for lipid of interest is plotted against the effect for the same variant on endometrial cancer risk. Dashed lines represent GSMR estimates.

Acknowledgements

The authors thank the many individuals who participated in the Endometrial Cancer Association Consortium studies and the numerous institutions and their staff who supported recruitment.

The iCOGS and OncoArray endometrial cancer analysis were supported by NHMRC project grants [ID#1031333 & ID#1109286] Funding for the iCOGS infrastructure came from: the European Community's Seventh Framework Programme under grant agreement no 223175 [HEALTH-F2-2009-223175] [COGS], Cancer Research UK [C1287/A10118, C1287/A 10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565], the National Institutes of Health [CA128978] and Post-Cancer GWAS initiative [1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 - the GAME-ON initiative], the Department of Defence [W81XWH-10-1-0341], the Canadian Institutes of Health Research [CIHR] for the CIHR Team in Familial Risks of Breast Cancer, Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. OncoArray genotyping of ECAC cases was performed with the generous assistance of the Ovarian Cancer Association Consortium (OCAC). We particularly thank the efforts of Cathy Phelan. The OCAC OncoArray genotyping project was funded through grants from the US National Institutes of Health (CA1X01HG007491-01, U19-CA148112, R01-CA149429 and R01-CA058598); Canadian Institutes of Health Research (MOP-86727); and the Ovarian Cancer Research Fund. CIDR genotyping for the Oncoarray was conducted under contract 268201200008I. OncoArray genotyping of the BCAC controls was funded by Genome Canada Grant GPH-129344, NIH Grant U19 CA148065, and Cancer UK Grant C1287/A16563.

Stage 1 and stage 2 case genotyping was supported by the NHMRC [ID#552402, ID#1031333]. Control data were generated by the Wellcome Trust Case Control Consortium (WTCCC), and a full list of the investigators who contributed to the generation of the data is available from the WTCCC website. We acknowledge use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02 - funding for this project was provided by the Wellcome Trust under award 085475. NSECG was supported by the EU FP7 CHIBCHA grant, Wellcome Trust Centre for Human Genetics Core Grant 090532/Z/09Z, and CORGI was funded by Cancer Research UK. We thank Nick Martin, Dale Nyholt and Anjali Henders for access to GWAS data from QIMR Controls. Recruitment of the QIMR controls was supported by the NHMRC. The University of Newcastle, the Gladys M Brawn Senior Research Fellowship scheme, The Vincent Fairfax Family Foundation, the Hunter Medical Research Institute and the Hunter Area Pathology Service all contributed towards the costs of establishing the Hunter Community Study. The WHI program is funded by the National Heart, Lung, and Blood Institute, the US National Institutes of Health and the US Department of Health and Human Services (HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C and HHSN271201100004C). This work was also funded by NCI U19 CA148065-01. This research has been conducted using the UK Biobank Resource under applications 5122 and 9797.

ANECS recruitment was supported by project grants from the NHMRC [ID#339435], The Cancer Council Queensland [ID#4196615] and Cancer Council Tasmania [ID#403031 and

ID#457636]. SEARCH recruitment was funded by a programme grant from Cancer Research UK [C490/A10124]. The Bavarian Endometrial Cancer Study (BECS) was partly funded by the ELAN fund of the University of Erlangen. The Hannover-Jena Endometrial Cancer Study was partly supported by the Rudolf Bartling Foundation. The Leuven Endometrium Study (LES) was supported by the Verelst Foundation for endometrial cancer. The Mayo Endometrial Cancer Study (MECS) and Mayo controls (MAY) were supported by grants from the National Cancer Institute of United States Public Health Service [R01 CA122443, P30 CA15083, P50 CA136393, and GAME-ON the NCI Cancer Post-GWAS Initiative U19 CA148112], the Fred C and Katherine B Andersen Foundation, the Mayo Foundation, and the Ovarian Cancer Research Fund with support of the Smith family, in memory of Kathryn Sladek Smith. MoMaTEC received financial support from a Helse Vest Grant, the University of Bergen, Melzer Foundation, The Norwegian Cancer Society (Harald Andersens legat), The Research Council of Norway and Haukeland University Hospital. The Newcastle Endometrial Cancer Study (NECS) acknowledges contributions from the University of Newcastle, The NBN Children's Cancer Research Group, Ms Jennie Thomas and the Hunter Medical Research Institute. RENDOCAS was supported through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet [numbers: 20110222, 20110483, 20110141 and DF 07015], The Swedish Labor Market Insurance [number 100069] and The Swedish Cancer Society [number 11 0439]. The Cancer Hormone Replacement Epidemiology in Sweden Study (CAHRES, formerly called The Singapore and Swedish Breast/Endometrial Cancer Study; SASBAC) was supported by funding from the Agency for Science, Technology and Research of Singapore (A*STAR), the US National Institutes of Health and the Susan G. Komen Breast Cancer Foundation.

The Nurses' Health Study (NHS) is supported by the NCI, NIH Grants Number UM1 CA186107, P01 CA087969, R01 CA49449, 1R01 CA134958, and 2R01 CA082838. The authors thank the participants and staff of the Nurses' Health Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. The authors also thank Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School. Finally, the authors also acknowledge Pati Soule and Hardeep Ranu for their laboratory assistance. The Connecticut Endometrial Cancer Study was supported by NCI, NIH Grant Number RO1CA98346. The Fred Hutchinson Cancer Research Center (FHCRC) is supported by NCI, NIH Grant Number NIH RO1 CA105212, RO1 CA 87538, RO1 CA75977, RO3 CA80636, NO1 HD23166, R35 CA39779, KO5 CA92002 and funds from the Fred Hutchinson Cancer Research Center. The Multiethnic Cohort Study (MEC) is supported by the NCI, NHI Grants Number CA54281, CA128008 and 2R01 CA082838. The California Teachers Study (CTS) is supported by NCI, NIH Grant Number 2R01 CA082838, R01 CA91019 and R01 CA77398, and contract 97-10500 from the California Breast Cancer Research Fund. The Polish Endometrial Cancer Study (PECS) is supported by the Intramural Research Program of the NCI. The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) is supported by the Extramural and the Intramural Research Programs of the NCI.

Melbourne Collaborative Cohort Study (MCCS) cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414 and 1074383 and by

infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database.