Adiposity and endometrial cancer risk in postmenopausal women: a sequential causal mediation analysis

S Ghazaleh Dashti,[1,2,3] Dallas R English,[1,4] Julie A Simpson,[1] Amalia Karahalios,[1,5] Margarita Moreno-Betancur, [3,6] Carine Biessy,[2] Sabina Rinaldi,[2] Pietro Ferrari,[2] Anne Tjønneland,[7] Jytte Halkjær,[7] Christina C Dahm,[8] Helene Tilma Vistisen,[8] Florence Menegaux,[9] Vittorio Perduca,[10] Gianluca Severi,[9,11,12] Krasimira Aleksandrova,[13,14] Matthias B. Schulze,[14,15] Giovanna Masala,[16] Sabina Sieri,[17] Rosario Tumino,[18] Alessandra Macciotta,[19] Salvatore Panico,[20] Anouk E. Hiensch,[21] Anne M. Ma,[21] J. Ramón Quirós,[22] Antonio Agudo,[23] Maria-Jose Sánchez,[24,25,26,27] Pilar Amiano,[28,29] Sandra Colorado-Yohar,[30,31,32] Eva Ardanaz,[33,34,35] Naomi E Allen,[36] Elisabete Weiderpass,[37] Renée Turzanski Fortner,[38] Sofia Christakoudi,[39,40], Konstantinos K. Tsilidis,[39,41] Elio Riboli,[39] Rudolf Kaaks,[38] Marc J Gunter,[2] Vivian Viallon,*[2] Laure Dossus,*[2]

*Joint senior authors

1 Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia
2 Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC), Lyon, France
3 Clinical Epidemiology and Biostatistics Unit, Murdoch Children's Research Institute, Melbourne, Australia
4 Cancer Epidemiology and Intelligence Division, Cancer Council Victoria, Melbourne, Victoria, Australia
5 School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia
6 Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia
7 Danish Cancer Society Research Center, Copenhagen Denmark
8 Department of Public Health, Aarhus University, Aarhus, Denmark
9 Université Paris-Saclay, UVSQ, CESP U1018 INSERM, Villejuif, France
10 Laboratoire de Mathématiques Appliquées à Paris 5—MAP5 (UMR CNRS 8145), Université Paris Descartes, Université de Paris, Paris, France
11 Gustave Roussy, Villejuif, France
12 Department of Statistics, Computer Science, Applications “G. Parenti”, University of Florence, Italy
13 Nutrition, Immunity and Metabolism Senior Scientist Group, Department of Nutrition and Gerontology, German Institute of Human Nutrition, Potsdam-Rehbruecke (DIfE), Nuthetal, Germany
14 University of Potsdam, Institute of Nutritional Science, Potsdam, German
15 Department of Moleculr Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, German
16 Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network - ISPRO, Florence, Italy
17 Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
18 Cancer Registry and Histopathology Department, Provincial Health Authority (ASP) Ragusa, Italy
19 Department of Clinical and Biological Sciences, University of Turin, Italy
20 Dipoartimento Di Medicina Clinica E Chirurgia Federico II University, Naples, Italy
21 Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
22 Public Health Directorate, Asturias, Spain
23 Unit of Nutrition and Cancer, Catalan Institute of Oncology - ICO, Nutrition and Cancer Group, Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
24 Escuela Andaluza de Salud Pública (EASP), Granada, Spain
25 Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain
26 Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
27 Universidad de Granada, Granada, Spain
28 Ministry of Health of the Basque Government, Public Health Division of Gipuzkoa, Biodonostia Health Research Institute, Donostia-San Sebastian, Spain
29 CIBER Epidemiología y Salud Pública, Madrid, Spain
30 Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain
31 CIBER Epidemiología y Salud Pública (CIBERESP), Spain.
32 Research Group on Demography and Health, National Faculty of Public Health, University of Antioquia, Medellín, Colombia
33 Navarra Public Health Institute, Pamplona, Spain
34 IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
35 CIBER Epidemiology and Public Health CIBERESP, Madrid, Spain
36 Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom
37 International Agency for Research on Cancer (IARC), Lyon, France

38 Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
39 Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, Norfolk Place, St Mary's Campus, London W2 1PG, United Kingdom
40 MRC Centre for Transplantation, King's College London, Great Maze Pond, London SE1 9RT, United Kingdom
41 Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece

Corresponding author:
S Ghazaleh Dashti
Centre for Epidemiology and Biostatistics
Melbourne School of Population and Global Health
Level 3, 207 Bouverie Street
The University of Melbourne VIC 3010 Australia
Email: seyedeh.dashti@unimelb.edu.au

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

Abstract

Background

Adiposity increases endometrial cancer (EC) risk, possibly through inflammation, hyperinsulinemia, and increasing estrogens. We aimed to quantify the mediating effects of adiponectin (anti-inflammatory adipocytokine); interleukin-6, interleukin-1-receptor antagonist, TNF-receptor-1 and -2, and C-reactive protein (inflammatory status biomarkers); C-peptide (hyperinsulinemia biomarker); free estradiol and estrone (estrogen biomarkers) in the adiposity-EC link in postmenopausal women.

Methods

We used data from a case-control study within the European Prospective Investigation into Cancer and Nutrition. Eligible women had not had cancer, hysterectomy, diabetes, did not use oral contraceptives or hormone therapy, and were postmenopausal at recruitment. Mediating pathways from adiposity to EC were investigated by estimating natural indirect (NIE) and direct (NDE) effects using sequential mediation analysis.

Results

The study included 163 cases and 306 controls. The adjusted odds ratio (OR) for EC for BMI≥30*vs.*18.5≤BMI<25kg/m^2 was 2.51 (95%CI 1.26–5.02). The ORs^{NIE} were 1.95 (1.01–3.74) through all biomarkers (72% proportion mediated (PM)) decomposed as: 1.35 (1.06–1.73) through pathways originating with adiponectin (33%PM); 1.13 (0.71–1.80) through inflammation beyond [the potential influence of] adiponectin (13%PM); 1.05 (0.88–1.24) through C-peptide beyond adiponectin and inflammation (5%PM); and 1.22 (0.89–1.67) through estrogens beyond preceding biomarkers (21%PM). The OR^{NDE} not through biomarkers was 1.29 (0.54–3.09). Waist circumference gave similar results.

Conclusion

Reduced adiponectin and increased inflammatory biomarkers, C-peptide, and estrogens mediated ~70% of increased odds of EC in women with obesity *vs.* normal weight.

Impact

If replicated, these results could have implications for identifying targets for intervention to reduce EC risk in women with obesity.

Introduction

Excess adiposity is an important risk factor for endometrial cancer (EC) (1). In 2012, 34% (90% confidence interval (CI) 32%–36%) of diagnosed EC cases were attributable to overweight and obesity (2). Disturbed adipocytokine production and inflammation, hyperinsulinemia, and sex-steroid hormones – are hypothesized to underly the adiposity-EC link (3). In women with obesity, adipose tissue secretes less adiponectin (an anti-inflammatory adipocytokine) and more inflammatory adipocytokines (4). This inflammatory status may have mitogenic, anti-apoptotic, and angiogenic effects (5,6); reduce insulin sensitivity (7); or dysregulate aromatase expression and increase estrogen levels (8). Insulin sensitivity may also be reduced through increased hepatic glucose production in response to excess free fatty acids (7). The resulting hyperinsulinemia and higher circulating insulin levels is causally linked to EC (9). It may have mitogenic effects; increase free insulin-like growth factor-1 (IGF-1) levels (10,11); increase aromatase activity via IGF-1, thus increase estrogen levels (8); or down-regulate sex-hormone-binding globulin (SHBG) production and increase bioavailable estrogens (12). Bioavailable estrogens, especially when unopposed by progesterone, (3) may increase EC risk through mitogenic effects in endometrial tissue (13).

Causal mediation analysis (14) can quantify the role of biological pathways involved in the effect of adiposity on EC risk. As highlighted, it is improbable that the influences of the involved biomarkers are siloed. When measures for multiple biomarkers are available, mediation analysis approaches should account for correlations between biomarkers (14). Failing to do so and assessing the mediating roles of biomarkers individually may result in biased estimation of the effect explained by the biomarkers (14).

We used data from a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) (15,16) to quantify the mediating roles of biomarkers representing inflammatory status, hyperinsulinemia, and estrogens in explaining the effect of adiposity on EC risk in postmenopausal women. We estimated path-specific mediated effects using a sequential causal mediation analysis approach that allowed us to take dependences between biomarkers into account (17). The analysis relied on an assumed causal ordering between the pathways (17), which was decided based on the existing

evidence as described above. Although adiposity increases pre- and postmenopausal EC risk (1), we excluded premenopausal women because the mechanisms may be different before and after menopause (18).

Methods

Established in 1992, EPIC is a cohort study including ~370,000 women recruited from ten European countries. Details of baseline data collection, blood sample collection and storage, ascertainment of cancers and follow-up are published (16). The present study made use of data from a nested case-control study that investigated associations between EC risk and sex-steroid hormones, metabolic factors, and inflammatory biomarkers measured in baseline blood samples, for which follow-up ended between December 1999 and November 2003 (15).

Selection of cases and controls

The eligibility criteria for the original case-control study included no history of hysterectomy or diagnosis of cancer (except keratinocyte skin cancer) and no use of oral contraceptives or hormone therapy at blood collection. It included 233 incident primary EC cases and 446 controls (incidence density sampling), matched on recruitment center, age and fasting status at blood draw, time of blood draw, menopausal status (15). We used the updated EPIC database for this study, in which one case was found to have prevalent cancer at recruitment, and seven were no longer classified as EC cases based on additional information on tumor histology collected through pathology reports. We excluded these women and their matched controls. Additionally, women who at recruitment were not postmenopausal (46 cases, 86 controls), had a history of diabetes (9 cases, 16 controls), had BMI<18.5kg/m^2 (1 case, 3 controls) were excluded from the analysis. Finally, rather than performing multiple imputation to handle missing confounder data (the method used for missing biomarker data) we excluded women who had missing values for any of the selected confounders (see *Confounder selection* for further detail) because they comprised a small proportion of all participants (6 cases, 17 controls; 5% of the participants).

Biomarkers

Measured biomarkers were adiponectin; interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra), tumor necrosis factor-α (TNF-α), TNF receptor 1 (TNFR-1), TNFR-2, and C-reactive protein (CRP) (biomarkers of inflammatory status); C-peptide (hyperinsulinemia biomarker); calculated free estradiol (proxy for bioavailable estradiol) and estrone (estrogen pathway biomarkers) (19-23). Free estradiol was calculated from SHBG and total estradiol (24). Details of biomarker measurements have been previously published and are summarized in **Supplementary Table 1** (19-23). Samples from matched cases and controls were analyzed within the same batch and technicians performing the assays were blinded to the case status. The German Cancer Research Center (Deutsches Krebsforschungszentrum, Heidelberg, Germany) performed assays for IL-1Ra, TNFR1, and TNFR2. Other biomarkers were measured at the International Agency for Research on Cancer (Lyon, France).

Statistical Analyses

We had three measures of adiposity: BMI, waist circumference, and waist-hip ratio. To assist with the interpretations of the mediated effect (see Mediation analysis below), translation to policy, and to relax the parametric assumption of linearity, in primary analyses these were considered as categorical variables. The cut-off values to categorize women were based on guidelines (BMI ≥18.5-<25, ≥25-<30, and ≥30 kg/m^2; waist circumference ≤80, >80-≤88, and >88 cm) or tertile cut-offs (waist-hip ratio .≤0.78, >0.78-≤0.84, >0.84) (25).

To remove the effects of batch, fasting status and time of blood draw, for each biomarker we (i) fitted a linear mixed-effects model to the log-transformed biomarker value for the controls with batch as a random effect, fasting status at blood draw and time of blood draw as fixed effects; then (ii) for all women, derived a normalized value by subtracting the difference between the predicted mean batch-specific values and the overall mean from the observed values.

Confounder selection: The sequential mediation analysis used (see *Mediation analysis*) relied on no unmeasured exposure-outcome, mediator-outcome, and exposure-mediator confounding. A causal diagram (**Figure 1**) was developed with reference to existing evidence (13,26-28) to identify these confounders (14). These potential confounders included age at recruitment, number of full-term pregnancies, age at menarche and menopause, history of oral contraceptive use and hormone therapy, smoking status, and physical activity. Age at menopause had 7% missing value and was not included as a confounder in the analysis because it was weakly correlated with the exposure and biomarkers ($r<|0.17|$). Every other variable had <3% missing values. As previously described, participants with missing confounder data were excluded from analysis.

Exposure-mediator associations: Geometric mean ratios (GMR) of biomarkers (and 95% CIs) in relation to adiposity measures were estimated using linear regression models applied to log-transformed data for the controls, with adjustment for potential confounders.

Exposure-outcome and mediator-outcome associations: In models that included the outcome, we broke the matching to avoid losing participants and included the matching variables as covariates. We grouped the 21 recruitment centers into regions to avoid creating combinations with sparse data. Age at recruitment was modelled as restricted cubic splines (2 degrees of freedom corresponding to 3 knots). The ORs and 95% CIs for the association between EC and adiposity measures and biomarkers were estimated from models that included confounders. Models for biomarkers included BMI and when applicable, other biomarkers that might have confounded the biomarker-outcome association. The linearity of mediator-outcome associations was checked using models with restricted cubic splines (2 degrees of freedom).

Mediation analysis with multiple mediators: Figure 1 guided the analysis. The association between each adiposity measure and EC (total effect (TE) (14)) was decomposed into a natural indirect effect (NIE) through all biomarkers, and a natural direct effect (NDE). When comparing women with obesity versus normal weight, the NIE could be interpreted as the average change in the EC incidence if all women had obesity and their biomarker level changed from what it would naturally be if they had obesity to what it

would naturally be if they had normal weight. The NIE captures the effect of obesity on the EC incidence exerted through obesity-induced alterations in biomarker levels. The NDE could be interpreted as the average change in the EC incidence, if all women had the biomarker level they would have naturally had when of normal weight, and they changed from having obesity to having normal weight. This effect captures the part of the effect of obesity on the EC incidence that operates through pathways other than the biomarkers assessed in the mediation analysis (see also **Supplementary Figure 1** and reference (14)). Assuming that reduced adiponectin and increased inflammation biomarkers levels preceded and potentially, but not necessarily, influenced C-peptide and estrogen levels, and C-peptide preceded and potentially influenced estrogens (7,8,29) we sequentially (17) decomposed the estimated NIE into NIEs through 1) pathways originating with reduced adiponectin and increased inflammation biomarkers; 2) C-peptide beyond the influences of adiponectin and inflammation biomarkers and; 3) and estrogens (free estradiol and estrone), beyond the influences of adiponectin, inflammation biomarkers and C-peptide. Assuming that adiponectin preceded and influenced inflammation biomarkers (29), we additionally decomposed the first NIE into NIEs through 1) pathways originating with reduced adiponectin and 2) inflammation biomarkers beyond the potential influence of adiponectin. **Supplementary Figure 1** shows interpretations of these effects.

The NIEs and NDE were estimated on the log(OR) scale using a regression-standardization approach (where the log(OR)s were standardized to the distribution of confounders for all eligible women in the EPIC (30)). The estimation was based on nine sequential linear regression models for the biomarkers (mediators), conditional on exposure, baseline confounders, and any preceding biomarker in the sequence and the following four models for the outcome: *(i)* logistic regression conditional on exposure, confounders, and adiponectin, *(ii)* logistic regression conditional on exposure, confounders, adiponectin, and inflammation biomarkers, *(iii)* logistic regression conditional on exposure, confounders, adiponectin, inflammation biomarkers, and C-peptide; and *(iv)* logistic regression conditional on exposure, confounder, adiponectin, inflammation biomarkers, C-peptide free estradiol, and estrone. In combination with coefficients estimated from models for the mediators, outcome model *i* estimates the NIE through pathways originating with

adiponectin; model *ii* estimates the NIE through reduced adiponectin and increased inflammation; models *i* and *ii* estimate the NIE through inflammation biomarkers beyond adiponectin; models *ii* and *iii* estimate the NIE through C-peptide beyond adiponectin and inflammation biomarkers; models *iii* and *iv* estimate the NIE through estrogens beyond adiponectin, inflammation biomarkers and C-peptide; and model *iv* to estimate the NDE and the NIE through all the biomarkers (see also **Supplementary Table 2** for further details) (17). The regression models for which the mediator was the dependent variable were limited to controls to take the case-control design of the study into account (30). The proportion mediated (PM) was calculated on the log(OR) scale as $\log(OR)^{NIE}/(\log(OR)^{NDE} + \log(OR)^{NIE})$) (14).

Missing data: Seventeen percent of women had missing biomarker data, which was multiply imputed based on chained equations with 20 iterations (31). The imputation models included all variables in the mediation analyses, and recruitment center, height, weight, and hip circumference as auxiliary variables. Within each imputed dataset, 1,000 bootstrap samples were used to estimate standard errors for the TE, NDE, and NIEs. These estimates were pooled using Rubin's rules to calculate the final estimates and 95% CI (32).

Sensitivity Analysis: We also repeated the mediation analyses with continuous adiposity measures, and after excluding cases diagnosed within two years post-recruitment.

All analyses were performed in Stata version 15.1 (33).

Results

The analytic dataset included 163 EC cases and 306 controls. Median age at EC diagnosis was 63 years (interquartile range 60–68) (**Table 1**). At baseline, compared with controls, a smaller proportion of cases had used oral contraceptives, were current smokers, had moderate/high physical activity; a larger proportion had used hormone therapy, had BMI≥30 kg/m^2 or waist circumference>88 cm. Compared with women with no missing data, a higher proportion of women with missing biomarker values were cases,

fasting >6 hours at blood draw, from Northern Europe, current smokers, or with BMI≥30 kg/m^2 (**Supplementary Table 3**).

Exposure-mediator associations: For BMI (≥30 *vs*. ≥18.5-<25 kg/m^2) positive associations were observed with IL-6, IL-1Ra, TNF-R1, TNF-R2, CRP, C-peptide, free estradiol and estrone. Of these, CRP demonstrated the strongest association (GMR 2.85; 95%CI 2.13–3.81). An inverse association was observed for adiponectin (GMR 0.77; 95%CI 0.67–0.88). There was no evidence for an association with TNF-α. Similar patterns were seen for waist circumference and waist-hip ratio (**Table 2**; **Supplementary Table 4** complete-case analysis results).

Exposure-outcome and mediator-outcome associations: An increased OR for EC was observed for BMI ≥30 *vs*. ≥18.5-<25 kg/m^2 (OR 2.94; 95%CI 1.71–5.06) and waist circumference >88 *vs*. ≤80 cm (OR 2.10; 95%CI 1.31–3.36). The evidence for an association between BMI ≥25 *vs*. ≥18.5-<25 kg/m^2, waist circumference >80-≤88 vs. ≤80 cm, and both categories of waist-hip ratio and odds of EC was weak (**Table 3**). Therefore, we did not attempt to decompose these in the mediation analysis.

An inverse association was observed between adiponectin and EC, (OR per doubling concentration 0.65; 95%CI 0.47–0.90), and a positive association for IL-1Ra (OR 1.14; 95%CI 1.00 to 1.29), and estrone (OR 2.03; 95%CI 1.33 to 3.09). There was no strong evidence for departure from linearity for any of the biomarker-outcome associations (**Table 3**; **Supplementary Table 5** complete-case analysis results).

Since we neither observed an association between adiposity measures and TNF-α (**Table 2**) nor between TNF-α and EC risk (**Table 3**), we did not include this biomarker in our mediation analysis.

Mediation analysis with multiple mediators: Approximately 72% of the association between BMI (≥30 *vs*. ≥18.5-<25 kg/m^2) and EC was mediated through all the biomarkers (OR^{NIE} 1.95; 95%CI 1.01–3.74). Following a further decomposition of this NIE, there was suggestion for a 46% PM through pathways originating with reduced adiponectin and increased inflammation biomarkers, 5% PM through C-peptide beyond [the potential influences] of adiponectin and inflammation biomarkers, and 21% PM through

estrogens beyond adiponectin, inflammation biomarkers, and C-peptide. A decomposition of the NIE through adiponectin and inflammation biomarkers indicated a 33% PM through pathways originating with adiponectin and 13% PM through inflammation biomarkers beyond adiponectin. The estimated OR^{NDE} not through any of the biomarkers was 1.29 (95%CI 0.54–3.09). The OR^{NIE} point estimates for inflammation biomarkers, C-peptide, and estrogens were suggestive of moderate to weak increase in EC OR, but the 95%CIs were wide and also included a decreased OR (**Table 4**; **Supplementary Table 6** complete-case analysis results).

Similarly, for waist circumference (>88 *vs.*≤80 cm), approximately 76% of the association was mediated through all biomarkers (the OR^{NIE} 1.73; 95%CI 1.04–2.90). There was evidence for an NIE through reduced adiponectin and increased inflammation biomarkers (61% PM), as well as through reduced adiponectin (38% PM). The point estimates were also indicative of 24% PM through inflammation biomarkers beyond adiponectin, 4% PM through C-peptide beyond adiponectin and inflammation biomarkers, and 10% PM through estrogens beyond adiponectin, inflammation biomarkers, and C-peptide. However, the 95% CIs around the OR^{NIE} for these estimates were wide and included a decreased OR. The OR^{NDE} not through any of the biomarkers was 1.19 (95%CI 0.59–2.41) (**Table 4, Supplementary Table 6** complete-case analysis).

Mediation patterns were similar for continuous exposures (**Supplementary Table 7**), and after excluding cases diagnosed within two years post-recruitment (**Supplementary Table 8**).

Discussion

In this study of postmenopausal women, reduced adiponectin and increased inflammation biomarkers, C-peptide, and estrogens mediated most (>70%) of the increased odds of EC in women with obesity compared with normal weight. In the sequential mediation analysis, the largest mediating effect was observed for pathways originating with adiponectin. Based on the point estimates, depending on the measure of adiposity used, the second most important pathway was either inflammation (waist circumference) or estrogens (BMI). Our study had a relatively small size and there was high uncertainty around the estimate of

the NDE, not allowing us to make definitive conclusions about the direction and magnitude of the part of the effect of obesity on EC not explained by biomarkers included in our analysis.

We believe this is the first study to investigate the mediating role of multiple biomarkers in the adiposity-EC association using formal mediation analysis. The sequential mediation analysis circumvented the assumption of no exposure-induced mediator-outcome confounding and permitted quantifying the indirect effect through all the biomarkers, and decomposing this into path-specific indirect effects without assuming the biomarkers were independent (17). The sequential mediation analysis relied on a presupposed causal ordering of the pathways, which was based on existing evidence (7,8,29).

We had measures for a range of biomarkers representing the pathways of interest. However, because this was secondary analysis of an existing study, we were limited to biomarkers that had been measured and these may not have fully captured the entire physiological impact of these pathways. For the insulin pathway, we had measures for IGF binding protein (IGFBP)-1 and IGFBP-2 but did not include them because they do not have a clear relationship with adiposity or EC (13). We also excluded women who had a history of diabetes at recruitment because long-term hyperglycemia might influence insulin production (34). This might have impacted the generalizability of our study findings to women with diabetes. The observed mediating effect through biomarkers might have also been influenced by the quality of the measurements (the intra-batch coefficient of variation (CV) ranged from 2.6% (C-peptide) to 15% (IL-1Ra, TNF-α), and the inter-batch CV from <8% (adiponectin, TNF-R1, TNF-R2) to 27.7% (IL-1IRA) (19-23), and temporal stability of biomarkers. Finally, an observed mediating role through a biomarker does not necessarily indicate that the biomarker has a causal effect on EC risk. For example, even assuming that our presupposed causal ordering of the biomarkers held (i.e. adiponectin potentially influenced other biomarker levels but not vice versa), we cannot conclude that a hypothetical intervention to increase adiponectin level in women with obesity would reduce their EC risk. The observed NIE through adiponectin might have, at least partly, been because this biomarker performed well at reflecting the inflammatory status in women with obesity, which in turn could have influenced cancer risk through various mechanisms.

We controlled for the known confounders of the associations between exposure, mediators, and outcome, but residual confounding, for example through other unmeasured or mismeasured biomarkers, cannot be ruled out. We were also limited in exploring the potential influence of unmeasured confounding on our results, because sensitivity analysis and bias correction approaches for settings with multiple mediators are not developed yet. Although our analysis assumed that it was adiposity that influenced biomarker levels, we cannot be certain about the temporal exposure-mediator ordering, because they were measured cross-sectionally. Preclinical cancers might have also influenced measures of adiposity and biomarkers. However, results from a sensitivity analysis that excluded cases diagnosed within two years post-recruitments were comparable to our primary analysis. We used a regression-based mediation analysis that could be adapted to the matched design (30). A limitation was that it did not allow including possible exposure-mediator and mediator-mediator interactions (17). Another limitation of our study was that biomarker measurements were missing for 17% of the participants and a comparison of women with and without missing data indicated that missingness was not completely at random. We multiply imputed these missing values, making an unverifiable assumption that missingness was at random, with missing data only depending on measured variables. Violation of this assumption would have introduced bias into our estimates of the NIE and NDE, but we attempted to reduce this possibility by defining multiple imputation models that included key variables (including BMI, waist circumference, and waist-hip ratio).

The predominant hypothesis in explaining the adiposity-EC association in postmenopausal women is increased estrogen production in adipose tissue (35,36). To explore the mediating role of estrogens, two studies assessed the degree to which adjusting for estradiol attenuated the BMI-EC association (19,37). The adjustment weakened but did not entirely explain the association (OR for BMI $\geq$30*vs.* <25kg/m^2 from 3.97 (95%CI 2.54-6.21) to 2.25 (95%CI 1.33-3.81) (37), and from 2.67 (95%CI 1.63-4.37) to 2.09 (95%CI 1.22-3.57) (19)), suggesting that other pathways are also likely at play. Similarly, we observed weak evidence of a small to moderate mediating effect through estrogens beyond the potential influence of preceding pathways.

Hyperinsulinemia might mediate the adiposity-EC association (3). However, we found almost no mediating effect through C-peptide beyond adiponectin and inflammation. Triglyceride glucose product (TyG) was used as a proxy for insulin resistance in a pooled study of six European cohorts. A negligible proportion (3.6%) of the total effect of BMI (≥30*vs.* ≥18.5-<25 kg/m^2; hazard ratio (HR) 2.61; 95%CI 2.29-2.99) was mediated by TyG (HR^{NIE} 1.04; 95%CI 0.99-1.09) (38). In both studies, there was little association between TyG or C-peptide and EC after adjusting for BMI. However, other studies have found associations between fasting insulin (9,39) and C-peptide (40)) and EC, after adjustment for BMI. Put together with the likely limitations of fasting and non-fasting C-peptide in capturing insulin resistance (39), such discrepancies warrant additional research to elucidate the mediating effect of the insulin resistance pathway.

In our analysis, the largest mediating effect was observed through reduced adiponectin and increased inflammation biomarkers. A further decomposition of this indirect effect suggested that a larger mediating effect was through adiponectin, with indication of a smaller mediating effect through inflammation beyond the potential influence of adiponectin. Existing evidence for inverse adiposity-adiponectin (41) and adiponectin-EC associations (42) support our observation. Based on a principal-component factor analysis, data from the original case-control study previously demonstrated a reduction in the OR for the association between BMI (≥30*vs.*<25 kg/m^2) and postmenopausal EC from 2.73 (95%CI, 1.66-4.50) to 1.65 (95%CI, 0.92-2.98) (~50% reduction on log(OR) scale) after adjusting for a factor with >|40%| loading for adiponectin, together with CRP, C-peptide, IGFBP-1, IGFBP-2, SHBG, and HDL cholesterol (15).

In summary, about 70% of the increased odds of EC risk in women with obesity compared with normal weight was mediated together through adiponectin, inflammation, C-peptide, and estrogens. We applied a novel mediation analysis approach to quantify the mediating effects through these pathways jointly, and the path-specific indirect effects. The applied method was able to handle multiple correlated biomarkers without assuming independence. Pathways originating with reduced adiponectin had the most important role in explaining the link. Future studies with larger sample sizes and a range of biomarkers reflecting the pathways, and preferably repeated measures for adiposity and biomarkers are needed to replicate findings from this study. Larger studies are needed to estimate the mediated effects with more

certainty and to allow exploring the possible influence of adiposity-biomarkers and biomarker-biomarker interactions on those effects. Ideally, as has been done in this study, future research in this area would take advantage of the advances in mediation analysis to properly account for the dependences between biomarkers.

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

Funding: This work was supported by the World Cancer Research Fund (grants 2003/18 and 2007/13); Australian National Health and Medical Research Council grants (1150591 to SGD and 1104975 to JAS); and Melbourne Research Scholarship (to SGD). MMB is the recipient of an Australian Research Council Discovery Early Career Award (project number DE190101326) funded by the Australian Government. The work reported in this paper was undertaken by SGD while hosted by the International Agency for Research on Cancer (IARC) and funded partially by a PhD Scholarship by IARC. The coordination of EPIC is financially supported by the European Commission and the International Agency for Research on Cancer. The national cohorts are supported by the following: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, and Institut National de la Santé et de la Recherche Médicale (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum, and Federal Ministry of Education and Research (Germany); Stavros Niarchos Foundation and Hellenic Health Foundation (Greece); Italian Association for Research on Cancer and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sport, Netherlands Cancer Registry, LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund, and Statistics Netherlands (the Netherlands); European Research Council and Nordforsk, Nordic Centre of Excellence Programme on Food, Nutrition and Health (Norway); Health Research Fund, Regional Governments of Andalucía, Asturias, Basque Country, Murcia, and Navarra, and Instituto de Salud Carlos III Red Temática de Investigación Cooperativa en Salud (Spain); Swedish Cancer Society, Swedish Scientific Council, and Regional Governments of Skåne and Västerbotten (Sweden); and Cancer Research UK, Medical Research Council, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and Wellcome Trust (United Kingdom).

Acknowledgements

We thank the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands for their contribution and ongoing support to the EPIC Study. This study also made use of data from the EPIC-Norfolk cohort.

References

1. Fader AN, Arriba LN, Frasure HE, von Gruenigen VE. Endometrial cancer and obesity: epidemiology, biomarkers, prevention and survivorship. Gynecol Oncol **2009**;114(1):121-7 doi 10.1016/j.ygyno.2009.03.039.

2. Arnold M, Pandeya N, Byrnes G, Renehan PAG, Stevens GA, Ezzati PM, *et al.* Global burden of cancer attributable to high body-mass index in 2012: a population-based study. Lancet Oncol **2015**;16(1):36-46 doi 10.1016/S1470-2045(14)71123-4.

3. Shaw E, Farris M, McNeil J, Friedenreich C. Obesity and Endometrial Cancer. Recent Results Cancer Res **2016**;208:107-36 doi 10.1007/978-3-319-42542-9_7.

4. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nat Rev Cancer **2015**;15(8):484-98 doi 10.1038/nrc3967.

5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell **2011**;144(5):646-74 doi 10.1016/j.cell.2011.02.013.

6. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, Inflammation, and Cancer. Annu Rev Pathol **2016**;11:421-49 doi 10.1146/annurev-pathol-012615-044359.

7. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? Curr Opin Endocrinol Diabetes Obes **2012**;19(2):81-7 doi 10.1097/MED.0b013e3283514e13.

8. Gerard C, Brown KA. Obesity and breast cancer - Role of estrogens and the molecular underpinnings of aromatase regulation in breast adipose tissue. Mol Cell Endocrinol **2017** doi 10.1016/j.mce.2017.09.014.

9. Nead KT, Sharp SJ, Thompson DJ, Painter JN, Savage DB, Semple RK, *et al.* Evidence of a Causal Association Between Insulinemia and Endometrial Cancer: A Mendelian Randomization Analysis. J Natl Cancer Inst **2015**;107(9) doi 10.1093/jnci/djv178.

10. Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol Metab **2006**;17(8):328-36 doi 10.1016/j.tem.2006.08.006.

11. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer **2008**;8(12):915-28 doi 10.1038/nrc2536.

12. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer **2004**;4(8):579-91 doi 10.1038/nrc1408.

13. Interventions IWGotEoC-P. Absence of excess body fatness, IARC Handbooks of Cancer Prevention ; Volume 16. **2018**.

14. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health **2016**;37:17-32 doi 10.1146/annurev-publhealth-032315-021402.

15. Dossus L, Lukanova A, Rinaldi S, Allen N, Cust AE, Becker S, *et al.* Hormonal, metabolic, and inflammatory profiles and endometrial cancer risk within the EPIC cohort--a factor analysis. Am J Epidemiol **2013**;177(8):787-99 doi 10.1093/aje/kws309.

16. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, *et al.* European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr **2002**;5(6B):1113-24 doi 10.1079/PHN2002394.

17. VanderWeele TJ, Vansteelandt S. Mediation Analysis with Multiple Mediators. Epidemiol Methods **2014**;2(1):95-115 doi 10.1515/em-2012-0010.

18. Merritt MA, Gunter MJ. Epidemiologic Evidence for the Obesity-Endometrial Cancer Relationship. Energ Balance Cancer **2018**;13:1-19 doi 10.1007/978-3-319-63483-8_1.

19. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, *et al.* Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer **2008**;15(2):485-97 doi 10.1677/ERC-07-0064.

20. Cust AE, Allen NE, Rinaldi S, Dossus L, Friedenreich C, Olsen A, *et al.* Serum levels of C-peptide, IGFBP-1 and IGFBP-2 and endometrial cancer risk; results from the European prospective investigation into cancer and nutrition. Int J Cancer **2007**;120(12):2656-64 doi 10.1002/ijc.22578.

21. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Lukanova A, *et al.* Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab **2007**;92(1):255-63 doi 10.1210/jc.2006-1371.

22. Dossus L, Becker S, Rinaldi S, Lukanova A, Tjonneland A, Olsen A, *et al.* Tumor necrosis factor (TNF)-alpha, soluble TNF receptors and endometrial cancer risk: the EPIC study. Int J Cancer **2011**;129(8):2032-7 doi 10.1002/ijc.25840.

23. Dossus L, Rinaldi S, Becker S, Lukanova A, Tjonneland A, Olsen A, *et al.* Obesity, inflammatory markers, and endometrial cancer risk: a prospective case-control study. Endocr Relat Cancer **2010**;17(4):1007-19 doi 10.1677/ERC-10-0053.

24. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, *et al.* Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev **2002**;11(10 Pt 1):1065-71.

25. World Health Organization. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. https://apps.who.int/iris/bitstream/handle/10665/44583/9789241501491_eng.pdf?sequence=1; 2008.

26. Dossus L, Allen N, Kaaks R, Bakken K, Lund E, Tjonneland A, *et al.* Reproductive risk factors and endometrial cancer: the European Prospective Investigation into Cancer and Nutrition. Int J Cancer **2010**;127(2):442-51 doi 10.1002/ijc.25050.

27. World Cancer Research Fund International/American Institute for Cancer Research. Continuous Update Project Report: Diet, Nutrition, Physical Activity and endometrial cancer. Available at: https://www.wcrf.org/dietandcancer/contents2018.

28. Cook, L., Weiss, N., Doherty, J., Chen, C. Endometrial Cancer. In (Ed.), Cancer Epidemiology and Prevention. : Oxford University Press,. Retrieved 7 Apr. 2019, from http://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780195149616.001.0001/acprof-9780195149616-chapter-53. **2006**.

29. Iyengar NM, Gucalp A, Dannenberg AJ, Hudis CA. Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. J Clin Oncol **2016**;34(35):4270-6 doi 10.1200/JCO.2016.67.4283.

30. VanderWeele TJ, Tchetgen Tchetgen EJ. Mediation Analysis With Matched Case-Control Study Designs. Am J Epidemiol **2016**;183(9):869-70 doi 10.1093/aje/kww038.

31. StataCorp LP. Stata multiple-imputation reference manual : release 12. College Station, Tex.: Stata Press; 2011. iii, 365 p. p.

32. Schomaker M, Heumann H. Bootstrap inference when using multiple imputation. Statistics in Medicine **2018**;37(14):2252-66 doi 10.1002/sim.7654.

33. Albert JM, Li Y, Sun J, Woyczynski WA, Nelson S. Continuous-time causal mediation analysis. Stat Med **2019**;38(22):4334-47 doi 10.1002/sim.8300.

34. Ferrannini E, Mari A. beta-Cell function in type 2 diabetes. Metabolism **2014**;63(10):1217-27 doi 10.1016/j.metabol.2014.05.012.

35. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev **2002**;11(12):1531-43.

36. Rodriguez AC, Blanchard Z, Maurer KA, Gertz J. Estrogen Signaling in Endometrial Cancer: a Key Oncogenic Pathway with Several Open Questions. Horm Cancer-Us **2019**;10(2-3):51-63 doi 10.1007/s12672-019-0358-9.

37. Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, *et al.* Serum Estrogens and Estrogen Metabolites and Endometrial Cancer Risk among Postmenopausal Women. Cancer Epidemiol Biomarkers Prev **2016**;25(7):1081-9 doi 10.1158/1055-9965.EPI-16-0225.

38. Fritz J, Bjorge T, Nagel G, Manjer J, Engeland A, Haggstrom C, *et al.* The triglyceride-glucose index as a measure of insulin resistance and risk of obesity-related cancers. Int J Epidemiol **2019** doi 10.1093/ije/dyz053.

39. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Manson JE, Li J, *et al.* A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. Cancer Epidemiol Biomarkers Prev **2008**;17(4):921-9 doi 10.1158/1055-9965.EPI-07-2686.

40. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, Micheli A, Arslan AA, Rinaldi S, *et al.* Prediagnostic levels of C-peptide, IGF-I, IGFBP -1, -2 and -3 and risk of endometrial cancer. Int J Cancer **2004**;108(2):262-8 doi 10.1002/ijc.11544.

41. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? Diabetes Care **2003**;26(8):2442-50 doi 10.2337/diacare.26.8.2442.

42. Yoon YS, Kwon AR, Lee YK, Oh SW. Circulating adipokines and risk of obesity related cancers: A systematic review and meta-analysis. Obesity research & clinical practice **2019**;13(4):329-39 doi 10.1016/j.orcp.2019.03.006.

1
2

Table 1 - Baseline characteristics of endometrial cancer cases and controls

	Controls N=306	Cases N=163
Age at blood collection, years; median (IQR)	60.0 (56.6-63.0)	60.4 (56.7-63.2)
Age at cancer diagnosis, years; median (IQR)	-	63 (60-68)
Follow-up time, years; median (IQR)	6 (4-7)	3 (2-5)
mean (SD)	5.6 (1.6)	3.5 (2.1)
Fasting status at blood draw, hours; n(%)		
<3 hours	154 (50)	85 (52)
3-6	56 (18)	27 (17)
>6	96 (31)	51 (31)
Region of recruitment*; n(%)		
Western Europe	77 (25)	43 (26)
Northern Europe	119 (39)	66 (40)
Southern Europe	110 (36)	54 (33)
Full-term pregnancies, number; median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.0)
Age at menarche, years; median (IQR)	13.0 (12.0-14.0)	13.0 (12.0-14.0)
Had history of oral contraceptive use; n(%)	109 (36)	47 (29)
Had history of hormone therapy; n(%)	47 (15)	36 (22)
Smoking status, n(%)		
never	196 (64)	112 (69)
former	54 (18)	33 (20)
current	56 (18)	18 (11)
Physical activity; n(%)		
Inactive to moderately inactive	106 (35)	64 (39)
moderately active to active	200 (65)	99 (61)
Body Mass Index, kg/m^2 ; n(%)		
≥18.5 & <25	129 (42)	47 (29)
≥25 & <30	121 (40)	61 (37)
≥30	56 (18)	55 (34)
median (IQR)	25.9 (23.5-28.5)	27.5 (24.0-32.4)
Waist Circumference, cm; n(%)		
≤80	122 (40)	54 (33)
>80 & ≤ 88	94 (31)	31 (19)
>88	90 (29)	78 (48)
median (IQR)	83.0 (76.0-91.0)	88.0 (78.5-95.5)
Waist-hip ratio; n(%)		
≤0.78	106 (35)	47 (29)
>0.78 & ≤0.84	110 (36)	56 (34)
>0.84	90 (29)	60 (37)
median (IQR)	0.8 (0.8-0.9)	0.8 (0.8-0.9)
Biomarkers; median (IQR)		
Adiponectin, µg/mL	10.87 (7.84-14.21)	8.94 (5.97-12.20)
Adiponectin missing value	1 (0)	0 (0)
Interleukin-6, pg/mL	1.2 (0.9-2.0)	1.4 (1.0-2.3)
Interleukin-6 missing value	11 (4)	9 (6)
Interleukin-1 receptor antagonist, pg/mL	22.4 (18.2-64.4)	35.6 (18.4-141.3)
Interleukin-1 receptor antagonist missing value	5 (2)	2 (1)
Tumor necrosis factor-α, pg/mL	1.0 (0.6-1.4)	1.0 (0.7-1.6)
Tumor necrosis factor-α missing value	3 (1)	1 (1)
Tumor necrosis factor-receptor 1, pg/mL	998.0 (912.1-1151.8)	1075.6 (920.0-1233.8)
Tumor necrosis factor-receptor 1 missing value	2 (1)	0 (0)
Tumor necrosis factor-receptor 2, pg/mL	1909.5 (1676.7-2187.8)	1988.4 (1722.1-2388.6)
Tumor necrosis factor-receptor 2 missing value	2 (1)	0 (0)
C-reactive protein, ng/mL	1345.6 (691.9-2640.7)	1745.1 (989.0-3223.7)
C-reactive protein missing value	7 (2)	8 (5)
C-peptide, ng/mL	3.1 (2.3-4.1)	3.4 (2.5-4.9)
C-peptide missing value	0 (0)	0 (0)
Calculated free estradiol, pg/mL	2.0 (1.6-2.5)	2.3 (1.8-3.2)
Free estradiol missing value	6 (2)	5 (3)
Estrone, pg/mL	32.7 (25.9-39.1)	35.7 (29.6-46.6)
Estrone missing value	23 (8)	15 (9)
Missing value for any of the biomarkers	47 (15)	32 (20)

* Centres from France, Germany, and the Netherlands were categorized as Western Europe; centres from Denmark, and the United Kingdom as Northern Europe; and centres from Italy, Spain, and Greece as Southern Europe.

Table 2 – exposure-mediator association; estimated ratios in geometric mean of biomarkers associated with body mass index and waist circumference; multiple imputation analyses limited to controls (n=306)

	Ratio of geometric means (95% confidence interval)					
	Body Mass Index, kg/m2		Waist circumference, cm		Waist-hip ratio	
Biomarker	≥25 vs. ≥18.5-<25	≥30 vs. ≥18.5-<25	>80-≤88 vs. ≤80	>88 vs. ≤80	>0.78-≤0.84 vs. ≤0.78	>0.84 vs. ≤0.78
Adiponectin	0.84 (0.76 to 0.94)	0.77 (0.67 to 0.88)	0.92 (0.83 to 1.03)	0.69 (0.61 to 0.78)	0.85 (0.76 to 0.95)	0.65 (0.58 to 0.74)
Interleukin-6	1.30 (1.12 to 1.52)	1.85 (1.53 to 2.26)	1.12 (0.95 to 1.31)	1.69 (1.43 to 2.00)	1.26 (1.07 to 1.49)	1.66 (1.39 to 1.99)
Interleukin-1 receptor antagonist	1.33 (1.01 to 1.74)	1.55 (1.09 to 2.20)	1.39 (1.04 to 1.85)	1.49 (1.10 to 2.01)	1.29 (0.96 to 1.73)	1.47 (1.08 to 2.01)
Tumor necrosis factor-α	1.16 (0.98 to 1.37)	1.12 (0.91 to 1.39)	1.02 (0.86 to 1.22)	1.08 (0.90 to 1.30)	1.12 (0.93 to 1.34)	1.14 (0.95 to 1.38)
Tumor necrosis factor-receptor 1	1.08 (1.03 to 1.14)	1.22 (1.14 to 1.30)	1.03 (0.97 to 1.09)	1.16 (1.10 to 1.23)	1.02 (0.97 to 1.08)	1.10 (1.03 to 1.17)
Tumor necrosis factor-receptor 2	1.07 (1.01 to 1.13)	1.19 (1.10 to 1.27)	1.02 (0.96 to 1.08)	1.12 (1.05 to 1.19)	1.02 (0.96 to 1.08)	1.07 (1.00 to 1.15)
C-reactive protein	1.54 (1.22 to 1.93)	2.85 (2.13 to 3.81)	1.41 (1.11 to 1.79)	2.57 (2.00 to 3.29)	1.46 (1.14 to 1.88)	2.28 (1.75 to 2.98)
C-peptide	1.29 (1.14 to 1.45)	1.45 (1.25 to 1.70)	1.21 (1.07 to 1.37)	1.56 (1.37 to 1.78)	1.18 (1.04 to 1.34)	1.51 (1.32 to 1.73)
Free estradiol	1.13 (1.03 to 1.25)	1.32 (1.16 to 1.49)	1.10 (1.00 to 1.22)	1.34 (1.21 to 1.49)	1.16 (1.05 to 1.29)	1.31 (1.17 to 1.47)
Estrone	1.06 (0.96 to 1.18)	1.17 (1.02 to 1.35)	0.95 (0.85 to 1.07)	1.14 (1.01 to 1.29)	0.9 9 (0.89 to 1.11)	1.11 (0.98 to 1.26)

Table 3 – estimated exposure-outcome and mediator-outcome associations; multiple imputation analysis

Body Mass Index, kg/m^2	≥25 vs. ≥18.5-<25			≥30 vs ≥18.5-<25		
OR (95% confidence interval)	1.44	(0.89 to	2.32)	2.94	(1.71 to	5.06)
Waist circumference, cm	>80-≤88 vs. ≤80			>88 vs ≤80		
OR (95% confidence interval)	0.69	(0.41 to	1.19)	2.10	(1.31 to	3.36)
Waist-hip ratio	>0.78-≤0.84 vs. ≤0.78			>0.84 vs ≤0.78		
OR (95% confidence interval)	1.13	(0.69 to	1.86)	1.57	(0.94 to	2.60)
Biomarker				Per doubling concentration		P value for evidence against linearity
Adiponectin						
Model 1 (adj for confounders + BMI)				0.65	(0.47 to	0.90) 0.05
Interleukin-6						
Model 1 (adj for confounders + BMI)				1.11	(0.87 to	1.43)
Model 2 (adj for confounders + BMI + adiponectin)				1.05	(0.82 to	1.36) 0.36
Interleukin-1 receptor antagonist						
Model 1 (adj for confounders + BMI)				1.15	(1.02 to	1.31)
Model 2 (adj for confounders + BMI + adiponectin)				1.14	(1.00 to	1.29) 0.57
Tumor necrosis factor-α						
Model 1 (adj for confounders + BMI)				0.99	(0.80 to	1.23)
Model 2 (adj for confounders + BMI + adiponectin)				0.97	(0.78 to	1.21) 0.83
Tumor necrosis factor-receptor 1						
Model 1 (adj for confounders + BMI)				1.05	(0.52 to	2.12)
Model 2 (adj for confounders + BMI + adiponectin)				1.05	(0.51 to	2.16) 0.30
Tumor necrosis factor-receptor 2						
Model 1 (adj for confounders + BMI)				0.97	(0.55 to	1.70)
Model 2 (adj for confounders + BMI + adiponectin)				0.97	(0.55 to	1.72) 0.24
C-reactive protein						
Model 1 (adj for confounders + BMI)				1.09	(0.92 to	1.29)
Model 2 (adj for confounders + BMI + adiponectin)				1.06	(0.89 to	1.25) 0.54
C-peptide						
Model 1 (adj for confounders + BMI)				1.16	(0.84 to	1.59)
Model 2 (adj for confounders + BMI + adiponectin + IL1-RA)				1.00	(0.72 to	1.40) 0.77
Free estradiol						
Model 1 (adj for confounders + BMI)				1.55	(1.08 to	2.24)
Model 2 (adj for confounders + BMI + adiponectin + IL1-RA + C-peptide)				1.38	(0.94 to	2.02) 0.11
Estrone						
Model 1 (adj for confounders + BMI)				2.13	(1.41 to	3.21)
Model 2 (adj for confounders + BMI + adiponectin + IL1-RA + C-peptide)				2.03	(1.33 to	3.09) 0.57

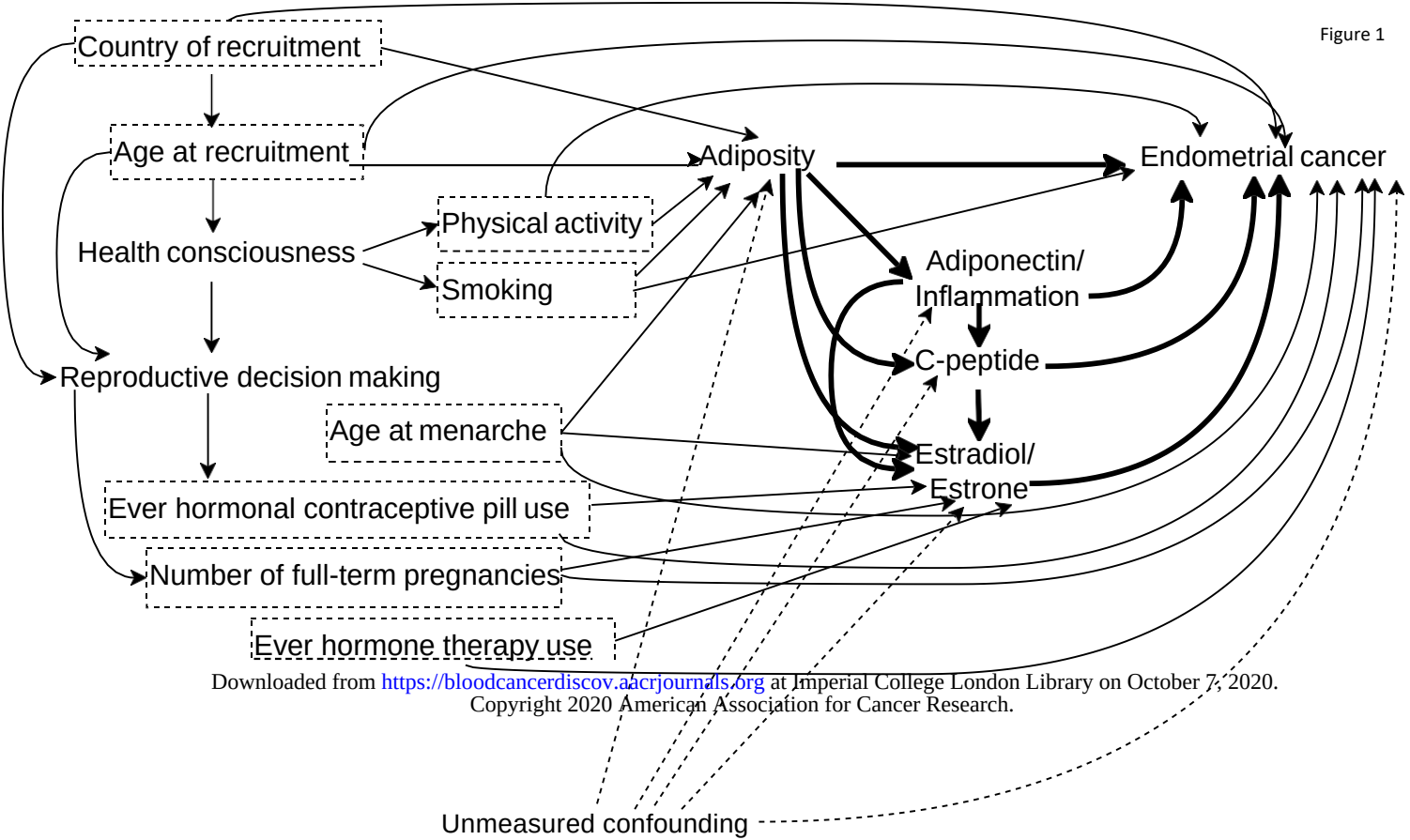
Table 4 - Estimated natural direct and indirect effects using sequential mediation analysis; analyses excluded women categorized as overweight; multiple imputation analysis

	Body Mass Index ≥30 vs. ≥18.5-<25 kg/m2				Waist Circumference >88 vs. ≤80 cm			
Number of cases/ number of controls	102/185				131/212			
	Odds Ratio (95% confidence interval)			% mediated on log odds scale	Odds Ratio (95% confidence interval)			% mediated on log odds scale
Total effect (estimated as the product of natural direct and indirect effects)	2.51	(1.26 to	5.02)		2.07	(1.20 to	3.55)	
Natural indirect effect through all the biomarkers	1.95	(1.01 to	3.74)	72%	1.73	(1.04 to	2.90)	76%
Natural indirect effect through reduced adiponectin and increased inflammation	1.53	(0.89 to	2.62)	46%	1.56	(1.01 to	2.42)	61%
Natural indirect effect through reduced adiponectin levels	1.35	(1.06 to	1.73)	33%	1.32	(1.03 to	1.68)	38%
Natural indirect effect through increased inflammation, beyond the potential influence of adiponectin	1.13	(0.71 to	1.80)	13%	1.19	(0.83 to	1.69)	24%
Natural indirect effect through increased c-peptide levels, beyond the potential influences of adiponectin and inflammation	1.05	(0.88 to	1.24)	5%	1.03	(0.89 to	1.19)	4%
Natural indirect effect through increased free estradiol and estrone levels, beyond the potential influences of adiponectin, inflammation, and c-peptide	1.22	(0.89 to	1.67)	21%	1.08	(0.88 to	1.33)	10%
Natural direct effect not through any of the biomarkers	1.29	(0.54 to	3.09)		1.19	(0.59 to	2.41)	

Figure legends

Figure 1- Assumed causal structure underlying the effect of adiposity on endometrial cancer
To avoid overloading the diagram, we did not include all the possible arrows between all the variables. We only included the arrows that were sufficient to flag a variable as a common cause (confounder) of either exposure-outcome, mediator-outcome, or exposure-mediator associations. Therefore, the diagram is not strictly a "causal diagram". The green arrows represent pathways (indirect and direct) that we were interested in. The red arrows represent the potentially biasing paths due to unmeasured confounding. Variables in the dashed boxed were included (conditioned on) in all multivariable analyses. Based on the assumed causal structure represented in this diagram, conditioning on none of these variables would have introduced collider bias.

Figure 1



Adiposity and endometrial cancer risk in postmenopausal women: a sequential causal mediation analysis

S. Ghazaleh Dashti, Dallas R. English, Julie A Simpson, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst October 2, 2020.