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

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15 **Conflict of interest:** The authors declare no potential conflicts of interest.  
16  
17

## 1 **Abstract**

### 2 **Background**

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

### 8 **Methods**

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

### 14 **Results**

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

### 22 **Conclusion**

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

### 25 **Impact**

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

28

## 1 **Introduction**

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

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

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

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

### 3 **Methods**

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

### 10 **Selection of cases and controls**

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

24

## 1 **Biomarkers**

2 Measured biomarkers were adiponectin; interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-  
3 1Ra), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF receptor 1 (TNFR-1), TNFR-2, and C-reactive protein (CRP)  
4 (biomarkers of inflammatory status); C-peptide (hyperinsulinemia biomarker); calculated free estradiol  
5 (proxy for bioavailable estradiol) and estrone (estrogen pathway biomarkers) (19-23). Free estradiol was  
6 calculated from SHBG and total estradiol (24). Details of biomarker measurements have been previously  
7 published and are summarized in **Supplementary Table 1** (19-23). Samples from matched cases and  
8 controls were analyzed within the same batch and technicians performing the assays were blinded to the  
9 case status. The German Cancer Research Center (Deutsches Krebsforschungszentrum, Heidelberg,  
10 Germany) performed assays for IL-1Ra, TNFR1, and TNFR2. Other biomarkers were measured at the  
11 International Agency for Research on Cancer (Lyon, France).

## 12 **Statistical Analyses**

13 We had three measures of adiposity: BMI, waist circumference, and waist-hip ratio. To assist with the  
14 interpretations of the mediated effect (see Mediation analysis below), translation to policy, and to relax the  
15 parametric assumption of linearity, in primary analyses these were considered as categorical variables. The  
16 cut-off values to categorize women were based on guidelines (BMI  $\geq 18.5$ - $<25$ ,  $\geq 25$ - $<30$ , and  $\geq 30$  kg/m<sup>2</sup>;  
17 waist circumference  $\leq 80$ ,  $>80$ - $\leq 88$ , and  $>88$  cm) or tertile cut-offs (waist-hip ratio  $\leq 0.78$ ,  $>0.78$ - $\leq 0.84$ ,  
18  $>0.84$ ) (25).

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

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

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

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

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



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

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

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

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

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

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

## 18 **Results**

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

1 fasting >6 hours at blood draw, from Northern Europe, current smokers, or with BMI $\geq$ 30 kg/m<sup>2</sup>

2 (**Supplementary Table 3**).

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

9 **Exposure-outcome and mediator-outcome associations:** An increased OR for EC was observed for  
10 BMI  $\geq$ 30 vs.  $\geq$ 18.5-<25 kg/m<sup>2</sup> (OR 2.94; 95%CI 1.71–5.06) and waist circumference >88 vs.  $\leq$ 80 cm (OR  
11 2.10; 95%CI 1.31–3.36). The evidence for an association between BMI  $\geq$ 25 vs.  $\geq$ 18.5-<25 kg/m<sup>2</sup>, waist  
12 circumference >80- $\leq$ 88 vs.  $\leq$ 80 cm, and both categories of waist-hip ratio and odds of EC was weak (**Table**  
13 **3**). Therefore, we did not attempt to decompose these in the mediation analysis.

14 An inverse association was observed between adiponectin and EC, (OR per doubling concentration  
15 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  
16 (OR 2.03; 95%CI 1.33 to 3.09). There was no strong evidence for departure from linearity for any of the  
17 biomarker-outcome associations (**Table 3; Supplementary Table 5** complete-case analysis results).

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

20 **Mediation analysis with multiple mediators:** Approximately 72% of the association between BMI  
21 ( $\geq$ 30 vs.  $\geq$ 18.5-<25 kg/m<sup>2</sup>) and EC was mediated through all the biomarkers (OR<sup>NIE</sup> 1.95; 95%CI 1.01–  
22 3.74). Following a further decomposition of this NIE, there was suggestion for a 46% PM through pathways  
23 originating with reduced adiponectin and increased inflammation biomarkers, 5% PM through C-peptide  
24 beyond [the potential influences] of adiponectin and inflammation biomarkers, and 21% PM through

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

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

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

## 19 **Discussion**

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

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

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

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

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

18 The predominant hypothesis in explaining the adiposity-EC association in postmenopausal women is  
19 increased estrogen production in adipose tissue (35,36). To explore the mediating role of estrogens, two  
20 studies assessed the degree to which adjusting for estradiol attenuated the BMI-EC association (19,37). The  
21 adjustment weakened but did not entirely explain the association (OR for BMI  $\geq 30$  vs.  $< 25$  kg/m<sup>2</sup> from 3.97  
22 (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-  
23 3.57) (19)), suggesting that other pathways are also likely at play. Similarly, we observed weak evidence of  
24 a small to moderate mediating effect through estrogens beyond the potential influence of preceding  
25 pathways.

1 Hyperinsulinemia might mediate the adiposity-EC association (3). However, we found almost no  
2 mediating effect through C-peptide beyond adiponectin and inflammation. Triglyceride glucose product  
3 (TyG) was used as a proxy for insulin resistance in a pooled study of six European cohorts. A negligible  
4 proportion (3.6%) of the total effect of BMI ( $\geq 30$  vs.  $\geq 18.5$ - $< 25$  kg/m<sup>2</sup>; hazard ratio (HR) 2.61; 95% CI 2.29-  
5 2.99) was mediated by TyG (HR<sup>NIE</sup> 1.04; 95% CI 0.99-1.09) (38). In both studies, there was little association  
6 between TyG or C-peptide and EC after adjusting for BMI. However, other studies have found associations  
7 between fasting insulin (9,39) and C-peptide (40)) and EC, after adjustment for BMI. Put together with the  
8 likely limitations of fasting and non-fasting C-peptide in capturing insulin resistance (39), such  
9 discrepancies warrant additional research to elucidate the mediating effect of the insulin resistance pathway.

10 In our analysis, the largest mediating effect was observed through reduced adiponectin and increased  
11 inflammation biomarkers. A further decomposition of this indirect effect suggested that a larger mediating  
12 effect was through adiponectin, with indication of a smaller mediating effect through inflammation beyond  
13 the potential influence of adiponectin. Existing evidence for inverse adiposity-adiponectin (41) and  
14 adiponectin-EC associations (42) support our observation. Based on a principal-component factor analysis,  
15 data from the original case-control study previously demonstrated a reduction in the OR for the association  
16 between BMI ( $\geq 30$  vs.  $< 25$  kg/m<sup>2</sup>) and postmenopausal EC from 2.73 (95% CI, 1.66-4.50) to 1.65 (95% CI,  
17 0.92-2.98) (~50% reduction on log(OR) scale) after adjusting for a factor with  $> |40\%|$  loading for  
18 adiponectin, together with CRP, C-peptide, IGFBP-1, IGFBP-2, SHBG, and HDL cholesterol (15).

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

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

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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 <sup>2</sup> ; 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.

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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)

Biomarker	Ratio of geometric means (95% confidence interval)					
	Body Mass Index, kg/m <sup>2</sup>		Waist circumference, cm		Waist-hip ratio	
	≥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- $\alpha$	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)	0.99 (0.89 to 1.11)	1.11 (0.98 to 1.26)

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

Body Mass Index, kg/m <sup>2</sup>	≥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
<b>Adiponectin</b>				
Model 1 (adj for confounders + BMI)		0.65 (0.47 to 0.90)		0.05
<b>Interleukin-6</b>				
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
<b>Interleukin-1 receptor antagonist</b>				
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
<b>Tumor necrosis factor-α</b>				
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
<b>Tumor necrosis factor-receptor 1</b>				
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
<b>Tumor necrosis factor-receptor 2</b>				
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
<b>C-reactive protein</b>				
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
<b>C-peptide</b>				
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
<b>Free estradiol</b>				
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
<b>Estrone</b>				
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

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

Number of cases/ number of controls	Body Mass Index ≥30 vs. ≥18.5-<25 kg/m <sup>2</sup>			Waist Circumference >88 vs. ≤80 cm			
	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)		

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1 Figure legends

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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.



