

1 **Metabolically-Defined Body Size Phenotypes and Risk of Endometrial Cancer in the European**
2 **Prospective Investigation into Cancer and Nutrition (EPIC)**

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66 The authors declare no potential conflicts of interest.
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71

72 **ABSTRACT**

73 **Background**

74 Obesity is a risk factor for endometrial cancer but whether metabolic dysfunction is associated with
75 endometrial cancer independent of body size is not known.

76 **Methods**

77 The association of metabolically-defined body size phenotypes with endometrial cancer risk was
78 investigated in a nested case-control study (817 cases/ 817 controls) within the European Prospective
79 Investigation into Cancer and Nutrition (EPIC). Concentrations of C-peptide were used to define
80 metabolically healthy (MH; <1st tertile) and metabolically unhealthy (MU; ≥1st tertile) status among
81 the control participants. These metabolic health definitions were combined with normal weight (NW;
82 Body Mass Index (BMI)<25kg/m² or Waist Circumference (WC)<80cm or Waist-to-Hip Ratio
83 (WHR)<0.8) and overweight (OW; BMI≥25kg/m² or WC≥80cm or WHR≥0.8) status, generating four
84 phenotype groups for each anthropometric measure: (1)MH/NW, (2)MH/OW (3)MU/NW and
85 (4)MU/OW.

86 **Results**

87 In a multivariable-adjusted conditional logistic regression model, compared with MH/NW
88 individuals, endometrial cancer risk was higher among those classified as MU/NW (OR_{WC}=1.48;
89 95%CI 1.05-2.10 and OR_{WHR}=1.68; 95%CI 1.21-2.35) and MU/OW (OR_{BMI}=2.38, 95%CI 1.73-3.27;
90 OR_{WC}=2.69, 95%CI 1.92-3.77 and OR_{WHR}=1.83, 95%CI 1.32-2.54). MH/OW individuals were also at
91 increased endometrial cancer risk compared to MH/NW individuals (OR_{WC}=1.94, 95%CI 1.24-3.04).

92 **Conclusions**

93 Women with metabolic dysfunction appear to have higher risk of endometrial cancer regardless of
94 their body size. However, overweight status raises endometrial cancer risk even among women with
95 lower insulin levels, suggesting that obesity-related pathways are relevant for the development of this
96 cancer beyond insulin.

97 **Impact**

98 Classifying women by metabolic health may be of greater utility in identifying those at higher risk for
99 endometrial cancer than anthropometry *per se*.

101 INTRODUCTION

102 Endometrial cancer is the second most common gynecological cancer worldwide, with 604,127 new
103 cases and 341,831 deaths reported in 2020 (1). Higher body mass index ($BMI \geq 25 \text{ kg/m}^2$) is a well-
104 established risk factor for endometrial cancer (2–5). A meta-analysis of prospective studies has shown
105 that every 5 kg/m^2 increase in BMI is associated with a 60% increase in endometrial cancer risk (6).
106 Recently, several studies have also shown that waist circumference (WC) and waist-to-hip ratio (WHR),
107 both indicators of central adiposity, may be associated with endometrial cancer risk independently of
108 BMI (7,8). Potential biological mechanisms linking obesity with endometrial cancer development
109 include alterations in the metabolism of endogenous hormones, such as sex steroids, insulin and
110 inflammation (9–11).

111 Hyperinsulinemia, a condition characterized by elevated levels of insulin in the fasting state, has been
112 positively associated with endometrial cancer risk in several prospective studies (12,13), and in a
113 Mendelian randomization analysis (5). C-peptide levels, a marker for pancreatic insulin secretion, have
114 also generally been associated with endometrial cancer risk (12,14). Mechanistically, insulin may
115 promote endometrial cancer development through direct mitogenic effects on the growth of
116 endometrial cancer cells, and indirectly via sex hormone disruption (15,16).

117 Metabolic dysfunction has been associated with a number of adverse health outcomes independent of
118 BMI (17–26). Indeed, over a third of adults in the normal weight range may have metabolic
119 dysfunction that puts them at elevated cardiometabolic disease risk (27). Accumulating evidence
120 suggests that individuals with metabolic dysfunction, either in the normal weight or overweight/obese
121 BMI range, are at greater risk of developing colorectal, breast, pancreatic, prostate and bladder
122 cancers, compared to subjects who are metabolically healthy (17,18,24,25,28). However, whether
123 metabolic dysregulation also raises endometrial cancer risk independent of obesity is less clear. A
124 study conducted within the Framingham Heart Study found that metabolic dysregulation (based on
125 elevated blood glucose) was associated with higher risk of endometrial cancer among women with
126 overweight and obesity, but not among women within the normal range of BMI and WHR (20).
127 However, another study in the SEER-Medicare linked database found that metabolic syndrome
128 (comprised of having three or more parameters out of clinical range including central obesity, fasting
129 glucose, blood pressure and triglycerides) remained associated with endometrial cancer even after
130 adjusting for level of obesity (29). However, to our knowledge no studies have specifically evaluated
131 hyperinsulinemia in association with endometrial cancer according to body size in a large-scale
132 prospective cohort.

133 To address these current gaps in the literature, we conducted an investigation of metabolically-defined
134 body size phenotypes (based on C-peptide levels combined with anthropometric measures) and their

135 association with endometrial cancer risk in a nested case-control study within the European
136 Prospective Investigation into Cancer and Nutrition (EPIC).

137 **MATERIALS AND METHODS**

138 **Study Population**

139 EPIC is an ongoing multicenter prospective cohort study designed to assess the relationship between
140 diet, lifestyle and genetic and metabolic factors with cancer and other chronic diseases. A detailed
141 description of the cohort has been published elsewhere (30,31). In summary, a total of 521,324
142 participants (~70% female) were recruited between 1992 and 2000 from 23 centers across ten
143 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain,
144 Sweden, and the United Kingdom). Written informed consent was provided by all participants. The
145 study was in accordance with human subjects' protection principles (Declaration of Helsinki) and was
146 approved by the ethical review boards from the International Agency for Research on Cancer (IARC)
147 and from all local centers.

148 **Follow-up and Ascertainment of Endometrial Cancer**

149 Incident endometrial cancer cases were identified using cancer registries in Norway, United Kingdom,
150 Spain, Italy, and the Netherlands and using a combination of sources such as active follow-up of study
151 subjects, cancer and pathology registries and health insurance records in France and Germany. The
152 collection and standardization of clinical and pathological data on each cancer site was performed
153 following a detailed protocol. The end of follow-up was established as the latest date of follow-up for
154 cancer incidence, death or end of follow-up, whichever came first. Censoring dates for complete
155 follow-up from cancer registries were between December 2009 and December 2013. Endometrial
156 cancer cases (C540-549) were identified using the 10th Revision of the International Classification of
157 Diseases ICD-10) and the 3rd Revision of the International Classification of Diseases for Oncology
158 (ICD-O-3). Endometrial cancer type 1 histologies included endometrioid adenocarcinoma,
159 adenosquamous carcinoma, adenocarcinoma with squamous metaplasia, adenocarcinoma not
160 otherwise specified, adenocarcinoma in adenomatous polyp, mucinous adenocarcinoma, mucin-
161 producing adenocarcinoma (codes 8380, 8560, 8570, 8140, 8210, 8480, 8481). The inclusion of
162 adenocarcinoma not otherwise specified in Type 1 is justified because endometrioid adenocarcinoma
163 is the most common type of adenocarcinoma. Type 2 histologies included squamous cell carcinoma,
164 clear cell adenocarcinoma, mixed cell adenocarcinoma, serous cystadenocarcinoma, papillary serous
165 cystadenocarcinoma (codes 8070, 8310, 8323, 8441, 8460). Other histologies were not classified into
166 either type (codes 8000, 8010, 8020, 8260, 8950, 8980).

167 **Selection of Case and Control Subjects**

168 Incident endometrial cancer cases were identified after the baseline blood collection and before the
169 end of the follow up in each study center. Women who had a previous cancer or had undergone
170 hysterectomy at the time of blood collection were excluded. For each case, one control participant was
171 randomly chosen from the overall EPIC cohort of women who were free of cancer at the time of
172 diagnosis of the index case. An incidence density sampling protocol for control selection was used,
173 such that controls could include participants who became a case later in time, while each control could
174 also be sampled more than once. The matching factors for cases and controls were study center,
175 fasting status, age at blood collection, time of day at blood collection (± 4 h), menopausal status,
176 exogenous hormone use and phase of menstrual cycle at blood collection.

177 **Laboratory Measurements**

178 Blood samples were collected at baseline according to standardised procedures and stored in the
179 central EPIC biorepository at IARC (-196°C , liquid nitrogen) for all countries included in this study.
180 C-peptide was measured in two phases. In the first phase, 378 serum samples were measured by an
181 immunoradiometric assay (Immunotech; Marseille, France), with intrabatch coefficients of variation
182 (CV) $<3\%$ and interbatch CVs $<11\%$ for a C-peptide concentration of 0.50 nmol/l (14). In the second
183 phase, 1256 plasma samples were measured by an ELISA assay (Mercodia; Uppsala, Sweden) with
184 intrabatch coefficients of variation (CV) $<7\%$ and interbatch CVs $<6\%$ for a C-peptide concentration
185 of 0.66 nmol/l (32). All measurements were performed in the immunoassay laboratory at IARC.
186 Samples from matched case-control sets were assayed in the same analytical batch. Laboratory
187 personnel were blinded to case-control status of the samples. Concentrations of C-peptide for cases
188 and controls by method of analysis are presented in Supplementary table 1.

189 **Assessment of Anthropometric, Lifestyle, and Dietary Exposures**

190 All participants underwent assessment of anthropometrics, lifestyle, dietary intake, medical history
191 and demographics at baseline. Standard protocols for the measurement of body weight and height
192 were used in all centres, except for Oxford, and Norway where these were self-reported. However,
193 previous studies have shown these self-reported anthropometric measures are valid for identifying
194 associations in epidemiological studies (33,34). Assessed weight and height were used to calculate
195 BMI (kg/m^2). Waist circumference (WC) was measured either at the narrowest torso circumference or
196 at the midpoint between the lower ribs and iliac crest. WC was divided by hip circumference to
197 generate the waist-to-hip ratio (WHR). Lifestyle and medical history self-reported questionnaires
198 collected information on education, smoking status, alcohol consumption, and physical activity level,
199 diabetes, and reproductive history (menopausal status, oral contraceptive use, menopausal hormone
200 use, age at menarche and menopause, and age and number of full-term pregnancies). The validated
201 Cambridge physical activity index was used to classify past-year physical activity levels in

202 occupational, leisure and household domains (35). Validated country/centre-specific dietary
203 questionnaires were used to obtain information on dietary intake. Different types of dietary
204 questionnaires were used in each study centre, including semi-quantitative food frequency
205 questionnaires (FFQ) with or without an estimation of individual average portion size and diet history
206 questionnaires combining a FFQ and 7-day dietary recalls (30,31).

207 **Metabolically defined body size phenotype definitions**

208 Concentrations of C-peptide amongst the control population were used to define metabolic health
209 status. Individuals were classified as metabolically healthy (MH) if below the first tertile
210 (Supplementary Table 2) or metabolically unhealthy (MU) if above the first tertile. This definition of
211 metabolic health was derived given that the risk of endometrial cancer was elevated in women in the
212 2nd and 3rd tertiles of C-peptide compared to those in the 1st tertile (Supplementary Table 3).
213 Additionally, the same procedure was performed using quartiles (1st quartile as metabolically healthy)
214 and median values (<median as metabolically healthy) of C-peptide standardized concentration
215 amongst the control population (Supplementary Table 2).

216 These metabolic health definitions were then combined with normal weight (NW; BMI<25 kg/m² or
217 WC< 80cm or WHR< 0.8) and overweight (OW; BMI≥25 kg/m² or WC≥ 80 cm or WHR≥ 0.8) status,
218 generating four phenotype groups for each of the three anthropometric measures separately (in total
219 12 groups (4x3)): metabolically healthy/normal weight (MH/NM); metabolically healthy/overweight
220 (MH/OW); metabolically unhealthy/normal weight (MU/NW) and metabolically
221 unhealthy/overweight (MU/OW). The WC and WHR cut-points were based on those from the
222 International Diabetes Federation (IDF)(36); which are gender and ethnic-specific cut-points for
223 European populations.

224 **Statistical analysis**

225 Descriptive analyses were performed and differences between cases and controls were assessed using
226 paired sample t-test for continuous variables and paired Chi-square test for categorical variables.
227 Descriptive analyses were also performed between metabolically defined body size phenotype groups
228 among the controls. As C-peptide was measured in two phases (in 2007 and then in 2019),
229 standardized values were used in the analysis. The standardisation was done by phase of the
230 measurements, with all features following the reduced, centered normal distribution (Mean=0 and
231 SD=1). Partial Pearson correlations in the control group adjusted for batch and age at blood collection,
232 between levels of C-peptide and anthropometrics variables were computed (Supplementary Table 4).
233 Conditional logistic regression, stratified by case-control set, was used to compute odds ratios (ORs)
234 and 95% confidence intervals (CIs) for the associations between metabolically-defined body size

235 phenotypes and endometrial cancer. The MH/NW was used as the reference category. The basic
236 model was built on matching factors only, while the adjusted model was built on matching factors and
237 a list of known risk factors for endometrial cancer which can potentially act as confounders,
238 including: age at menopause (age at menopause < 50; \geq 50 years; missing), age at menarche
239 (continuous), parity (0; 1; 2; $>$ 2; missing), hormone use (yes; no; missing), physical activity index
240 (inactive; moderately inactive; moderately active; active; missing), smoking status (never; former
241 smoker and current smoker; unknown), educational level (primary/no schooling;
242 technical/professional/secondary and longer education; missing), total energy intake (continuous),
243 alcohol intake (continuous), height (continuous) and diabetes (yes; no; missing). A separate model
244 including only overweight participants and with the MU/OW category as reference was also run. As
245 sensitivity analyses, all models were rerun using the phenotypes defined based on quartiles or on
246 median level of C-peptide cut points. Also, analyses were repeated considering only the upper tertile
247 as metabolically unhealthy. Sensitivity analyses were also performed among postmenopausal women
248 only; among non-exogenous hormone users only; among fasting participants only; among endometrial
249 cancer type 1 only (defined by histology as explained in case ascertainment section); and among
250 individuals from phase 2 only (as explained in laboratory measurements section). Further, sensitivity
251 analyses were conducted excluding cases diagnosed within the first 2 y of follow-up and their
252 matched controls and excluding participants with diabetes. Statistical tests used in the analysis were
253 all two-sided, and a p -value of <0.05 was considered statistically significant. Analyses were
254 conducted using SAS software.

255 **Data Availability**

256 EPIC data and biospecimens are available for investigators who seek to answer important questions
257 on health and disease in the context of research projects that are consistent with the legal and ethical
258 standard practices of IARC/WHO and the EPIC Centres. The primary responsibility for accessing the
259 data belongs to IARC and the EPIC centres. Access to materials from the EPIC study can be
260 requested by contacting epic@iarc.fr.

261 **RESULTS**

262 The current analysis used data from 1,634 women who were included in a nested case–control study
263 with available C-peptide levels. A total of 817 women were classified as incident endometrial cancer
264 cases and 817 were classified as matched controls. Among the cases, a total of 728 women were
265 classified as type 1, 40 women were classified as type 2 and 49 women had unknown tumour type.

266 **Table 1** shows that endometrial cancer cases had older age at menopause, but younger age at first
267 menstrual period and lower number of full-term pregnancies than the controls. Endometrial cancer

268 cases also had higher levels of C-peptide and greater BMI and WC than controls. In line with this, a
269 higher proportion of control participants were classified as MH/NW and MH/OW compared to cases
270 considering all anthropometric cut-points. The baseline characteristics of control group participants by
271 metabolically defined body size phenotypes are shown in **Table 2**. Compared to the MH/NW group
272 and considering the BMI classification, a greater proportion of MU/NW control participants reported
273 having longer education, higher alcohol intake and greater prevalence of current smoking and were
274 less frequently classified as physically active. In contrast to this, control participants in the MU/OW
275 group (considering the BMI classification) were less likely to be current smokers and to have longer
276 education, reported lower alcoholic intake and were more frequently classified as physically active
277 than MH/OW. It is important to note that around 40% of the controls were classified in the MU/OW
278 group while only around 11% were classified in the MH/OW group. The results based on WC and
279 WHR were broadly like the ones based on BMI.

280 The results for the associations between metabolically defined body size phenotypes and endometrial
281 cancer risk when adjusted for potential cofounders are described below by the phenotype categories
282 (**Table 3**).

283 *Metabolically healthy/overweight*

284 When using BMI and WHR cut-points, participants classified as MH/OW were at a higher risk of
285 endometrial cancer compared to MH/NW participants, albeit the associations were not statistically
286 significant ($OR_{BMI}=1.40$; 95%CI 0.91-2.15 and $OR_{WHR}=1.17$, 95%CI 0.75-1.81) and were at a
287 statistically significant lower risk of endometrial cancer than their MU/OW counterparts
288 ($OR_{BMI}=0.44$; 95%CI 0.26-0.74 and $OR_{WHR}=0.43$, 95%CI 0.25-0.76). In contrast, when using WC
289 cut-points, MH/OW women were at statistically significant higher risk of endometrial cancer
290 compared to MH/NW participants ($OR=1.94$, 95%CI 1.24-3.04) and they were at lower risk of
291 endometrial cancer compared to the MU/OW ($OR=0.80$; 95%CI 0.49-1.31), although the association
292 was not statistically significant.

293 *Metabolically unhealthy/normal weight*

294 MU/NW were at statistically significant higher risk of endometrial cancer than their MH/NW
295 counterparts when using WC ($OR=1.48$; 95%CI 1.05-2.10) and WHR ($OR=1.68$; 95%CI 1.21-2.35)
296 cut-points, while the results for the BMI cut-points were non-significant ($OR=1.16$, 95% CI 0.82-
297 1.64).

298 *Metabolically unhealthy/overweight*

299 MU/OW participants were at statistically significant higher risk of endometrial cancer compared to
300 MH/NW participants considering BMI (OR=2.38, 95%CI 1.73-3.27), WC (OR=2.69, 95%CI 1.92-
301 3.77) and WHR (OR=1.83, 95%CI 1.32-2.54) cut-points.

302 *Sensitivity analyses*

303 Similar results were observed when excluding cases diagnosed within the first 2 years of follow-up,
304 excluding individuals with diabetes, as well as when the analyses were restricted to individuals with
305 type 1 endometrial cancer or restricted to phase 2 samples (Supplementary Table 5). The results
306 restricted to non-exogenous hormone users and to fasting subjects were also broadly similar, however
307 most of the results were not statistically significant due to the reduced sample size (Supplementary
308 Table 5). Exclusion of pre-menopausal participants did not lead to substantial changes in the study
309 results for BMI cut-off points, but a few changes were observed for WC and WHR cut-points
310 (Supplementary Table 5). Sensitivity analyses also showed similar results when using C-peptide
311 quartiles and median cut-off points to define the metabolic health body size phenotypes
312 (Supplementary Table 6). Additionally, results defining the upper tertile as the metabolically
313 unhealthy group mirrored the main findings (Supplementary Table 7).

314 **DISCUSSION**

315 In this prospective analysis of metabolic health and endometrial cancer risk, metabolically unhealthy
316 normal weight and overweight participants, defined by C-peptide levels, were at higher endometrial
317 cancer risk compared to metabolically healthy normal weight women. In addition, metabolically
318 healthy overweight women were at higher endometrial cancer risk compared to metabolically healthy
319 normal weight women. These results indicate women with higher levels of insulin are at elevated risk
320 of endometrial cancer regardless of their body size, however, being overweight raises endometrial
321 cancer risk regardless of insulin profile.

322 Many, but not all, prior studies have shown a similar pattern of results for the relationships of
323 metabolically defined body size phenotypes with cardiovascular disease, type 2 diabetes, all-cause
324 mortality, open-angle glaucoma and obesity-related cancers (17–26,28,37,38). Our results lend further
325 support to the notion that, even though higher body size metrics are associated with increased
326 endometrial cancer risk, the assessment of metabolic dysfunction regardless of body size may be an
327 additional tool for risk stratification. Importantly, the study showed that normal weight women with
328 metabolic dysfunction have elevated risk for endometrial cancer. The potential mechanisms
329 underlying this relationship may involve the direct effect of insulin on normal endometrial and
330 malignant cells, as the insulin receptor is commonly expressed in the tumor cells (39). However,

331 multiple other factors may occur downstream of insulin signaling to impact endometrial
332 tumorigenesis, such as chronic inflammation and sex hormone disruption (10,15,16,40).

333 The factors influencing the development of metabolic dysfunction have been investigated and several
334 hypotheses have been proposed, including differences in body fat distribution, poor diet and physical
335 inactivity, and chronic inflammation (21,41–43). It has been suggested that individuals with metabolic
336 dysfunction tend to have higher intakes of sugar, sugar-sweetened beverages, and saturated fat as well
337 as lower intakes of fruits, whole grains, and protein from vegetable sources compared to metabolically
338 healthy individuals (21). On the other hand, metabolically healthy individuals tend to spend more time
339 in moderate to vigorous physical activities and less time in sedentary activities compared to
340 metabolically unhealthy individuals (41,44). Adipose tissue biology and function, including the
341 genetic determinants of body fat distribution, depot-specific fat metabolism, adipose tissue plasticity
342 and, particularly, adipogenesis also play a role (42). However, more research is needed to better
343 understand the mechanisms underlying the development of metabolic dysfunction, including the
344 potential role of the gut microbiota (42).

345 In the current analysis, individuals with overweight or obesity, regardless of their metabolic health
346 status, were at elevated endometrial cancer risk compared with MH/NW individuals. This is in line
347 with previous results from the EPIC cohort showing that obesity (including higher WC and WHR)
348 was associated with higher endometrial cancer risk compared to normal weight individuals (4). The
349 results for the WC-specific cut-off point were stronger and more consistent compared to the other
350 anthropometric cut-off points. These findings suggest that greater abdominal fat accumulation may
351 impact endometrial cancer risk irrespective of insulin levels. A potential pathway underlying this
352 relationship may include higher levels of oestrogen that are synthesized with greater abdominal fat in
353 both premenopausal (45) and postmenopausal women (46) given that higher exposure to unopposed
354 oestrogen is an established risk factor for endometrial cancer (47–50). Adipocyte hypertrophy and
355 hyperplasia stimulated pro-inflammatory immune response, chronic fibrosis and vascular
356 inflammation are also potential mechanisms that create a microenvironment conducive to
357 carcinogenesis (47,51).

358 To our knowledge, this is the first investigation of metabolically-defined body size phenotypes based
359 on C-peptide levels and endometrial cancer risk in a prospective cohort setting. The long-term follow-
360 up and high number of incident endometrial cancer cases recorded is a major strength of this study.
361 However, some limitations of the current study should also be considered. First, although there is no
362 universal definition of “metabolic health”, the analysis used only C-peptide levels as a marker of
363 metabolic health while there are more than 30 other possible definitions that have been used in
364 different studies, including homeostatic model assessment of insulin resistance (HOMA-IR) (using
365 insulin and glucose measures) (21,43). C-peptide may be a better indicator for long-term insulin

366 secretion than measuring insulin levels owing to its longer half-life (52). In the current study
367 hyperinsulinemia was defined based on tertiles of C-peptide level in controls, which was supported by
368 the results for the association between C-peptide tertiles and endometrial cancer risk showing elevated
369 risk for the upper two tertiles. This methodology has also been used in previous EPIC studies
370 classifying individuals according to their metabolically-defined body sized phenotypes (17). Further,
371 analyses that used quartiles and median of C-peptide levels showed a similar pattern of results.
372 However, future studies should aim to define clinically relevant cut-off points for normal C-peptide
373 levels, that can potentially be used for stratification for endometrial cancer risk. Finally, results from
374 the current study are largely applicable to white European women and future studies should
375 investigate other populations, such as black women who tend to have worse prognosis from
376 endometrial cancer (53,54).

377 In conclusion, we have shown that women with metabolic dysfunction appear to have higher risk of
378 endometrial cancer regardless of their body size. Therefore, it is possible that using only
379 anthropometric measurements to identify women at higher risk of endometrial cancer would exclude
380 normal-weight individuals with poor metabolic health and could underestimate the risk amongst
381 overweight individuals with hyperinsulinaemia. Normal weight and metabolically unhealthy women
382 represented 20 to 30% of the current sample, therefore this proportion of women would be missed
383 when using only body size for identifying women at higher risk of endometrial cancer. Thus,
384 classifying populations by metabolically defined body size phenotypes may be of greater utility in
385 identifying individuals at higher risk for endometrial cancer who would not have otherwise been
386 identified solely by anthropometric measures. Our findings also showed that overweight status may
387 raise endometrial cancer risk even among women with lower insulin levels, suggesting obesity-related
388 pathways are important for this cancer beyond insulin. The combination of anthropometric measures
389 with metabolic parameters, such as C-peptide, may allow more precise identification of the strata of
390 the population at greater endometrial cancer risk, which could be targeted for prevention strategies.

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588 **Table 1. Baseline characteristics of participants in a nested case control study within the**
 589 **European Prospective Investigation into Cancer and Nutrition (EPIC)**

Baseline Characteristics	Endometrial Cancer		p-value [#]
	Controls (N=817) Mean (SD) or N (%)	Cases (N=817) Mean (SD) or N (%)	
C-peptide (ng/ml)[^]	1.89 (1.22)	2.14 (1.43)	<.0001
Height (cm)	161.0 (7.0)	160.7 (6.8)	0.34
Body Mass Index (kg/m²)	25.7 (4.1)	27.7 (5.3)	<.0001
Waist circumference (cm)	81.3 (10.5)	85.3 (12.4)	<.0001
Waist/Hip Ratio (cm/cm)	0.8 (0.1)	0.8 (0.1)	0.05
Age at blood collection (years)	54.8 (7.6)	54.8 (7.6)	0.44
Fasting status at blood collection			0.99
Not fasting	366 (44.8%)	367 (44.9%)	
In between	148 (18.1%)	146 (17.9%)	
Fasting	303 (37.1%)	304 (37.2%)	
Age at menopause (years)	49.6 (4.3)	50.9 (4.0)	<.0001
Age at first menstrual period (years)	13.1 (1.6)	12.9 (1.5)	0.0017
Full term pregnancy			0.0034
Yes	707 (87.9%)	660 (82.8%)	
Number of full-term pregnancies*	2.4 (1.1)	2.3 (1.0)	0.02
Age at first full-term pregnancy (years)*	25.2 (4.2)	25.1 (4.1)	0.76
Menopausal status at blood collection			NA
Premenopausal	206 (25.2)	206 (25.2)	
Postmenopausal + Surgical postmen (bilateral ovariectomy)	496 (60.7)	496 (60.7)	
Perimenopausal	115 (14.1)	115 (14.1)	
Use of pill/HRT at blood collection			NA
No	650 (81.0)	650 (81.0)	
Yes	152 (19.0)	152 (19.0)	
Educational level			0.14
Primary/no schooling	365 (46.6%)	337 (43.4%)	
Technical/professional/secondary	277 (35.4%)	310 (39.9%)	
Longer education	141 (18.0%)	129 (16.6%)	
Physical activity			0.15
Inactive	201 (24.6%)	235 (28.8%)	
Moderately inactive	304 (37.2%)	270 (33.0%)	
Moderately active	190 (23.3%)	178 (21.8%)	
Active	108 (13.2%)	113 (13.8%)	
Smoking status			0.11
Never	495 (60.6%)	516 (63.2%)	
Former smoker	167 (20.4%)	173 (21.2%)	
Current smoker	138 (16.9%)	108 (13.2%)	
Diabetes			0.25
Yes	24 (3.4%)	32 (4.5%)	
Alcohol intake (g/d)[∞]	7.2 (10.5)	6.6 (9.8)	0.32
Total energy intake (kcal/d)	1918.3 (531.8)	1905.7 (591.7)	0.6
Metabolic health/BMI definition			<.0001
Metabolically healthy/normal weight ¹	179 (21.9%)	121 (14.8%)	
Metabolically healthy/overweight ²	94 (11.5%)	81 (9.9%)	
Metabolically unhealthy/normal weight ³	228 (27.9%)	166 (20.3%)	
Metabolically unhealthy/overweight ⁴	316 (38.7%)	449 (55.0%)	
Metabolic health/WC definition			<.0001
Metabolically healthy/normal weight ¹	180 (23.7%)	110 (14.5%)	
Metabolically healthy/overweight ²	84 (11.1%)	83 (10.9%)	
Metabolically unhealthy/normal weight ³	205 (27.0%)	169 (22.3%)	
Metabolically unhealthy/overweight ⁴	290 (38.2%)	397 (52.3%)	
Metabolic health/WHR definition			0.0006
Metabolically healthy/normal weight ¹	173 (22.8%)	125 (16.5%)	
Metabolically healthy/overweight ²	91 (12.0%)	68 (9.0%)	
Metabolically unhealthy/normal weight ³	207 (27.3%)	225 (29.6%)	
Metabolically unhealthy/overweight ⁴	288 (37.9%)	341 (44.9%)	

590 **Note.** BMI=Body Mass Index. WC=Waist Circumference. WHR=Waist-to-Hip ratio. HRT=hormone replacement therapy.
 591 NA=Not applicable since was used as a matching factor. [#]Paired sample t-test for continuous variable and paired Chi-square
 592 test for categorical variables. *Among parous women. ¹Metabolically healthy/normal weight (BMI < 25 kg/m² or Waist

593 circumference <80 cm or Waist-to-hip ratio <0.8) plus below tertile 1 of C-peptide. ²Metabolically healthy/overweight (BMI
594 ≥ 25 kg/m², or Waist circumference ≥ 80 cm or Waist-to-hip ratio ≥ 0.8), plus below tertile 1 of C-peptide. ³Metabolically
595 unhealthy/normal weight (BMI < 25 kg/m² or Waist circumference <80cm or Waist-to-hip ratio <0.8), plus above tertile 1 of
596 C-peptide. ⁴Metabolically unhealthy/overweight (BMI ≥ 25 kg/m², or Waist circumference ≥ 80 cm or Waist-to-hip ratio
597 ≥ 0.8), plus above tertile 1 of C-peptide. [^]Median (Interquartile range) among controls: 1.57 (1.05 – 2.32) and cases: 1.75
598 (1.16 – 2.64). [∞] Median (Interquartile range) among controls: 2.5 (0.3 – 10.8) and cases: 2.1 (0.2 – 9.3).

Table 2. Baseline characteristics of control group participants by metabolic health (hyperinsulinaemia) – defined body size phenotypes using anthropometric cut-points in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Baseline Characteristics	Metabolic health/BMI definition (N=1634)				p	Metabolic health/WC definition (N=1518)				p	Metabolic health/WHR definition (N=1518)				p
	Metabolically healthy		Metabolically unhealthy			Metabolically healthy		Metabolically unhealthy			Metabolically healthy		Metabolically unhealthy		
	NW ¹	OW/OB ²	NW ³	OW/OB ⁴		NW ¹	OW/OB ²	NW ³	OW/OB ⁴		NW ¹	OW/OB ²	NW ³	OW/OB ⁴	
N	300	175	394	765		290	167	374	687		298	159	432	629	
Age at blood collection (y)^a	53.2 (8.0)	54.5 (6.9)	54.6 (7.9)	55.6 (7.3)	<.001	52.9 (7.7)	55.1 (7.6)	54.2 (8.0)	56.2 (7.5)	<.001	52.9 (7.8)	55.3 (7.3)	54.5 (8.0)	56.1 (7.5)	<.001
Fasting status^b					<.001					<.001					<.001
Not fasting	73 (24.3)	34 (19.4)	268 (68.0)	358 (46.8)		67 (23.1)	31 (18.6)	238 (63.6)	311 (45.3)		70 (23.5)	28 (17.6)	277 (64.1)	272 (43.2)	
In between	60 (20.0)	38 (21.7)	65 (16.5)	131 (17.1)		59 (20.3)	30 (18.0)	58 (15.5)	117 (17.0)		62 (20.8)	27 (17.0)	63 (14.6)	112 (17.8)	
Fasting	167 (55.7)	103 (58.9)	61 (15.5)	276 (36.1)		164 (56.6)	106 (63.5)	78 (20.9)	259 (37.7)		166 (55.7)	104 (65.4)	92 (21.3)	245 (39.0)	
Age at menopause (y)^a	50.4 (3.8)	49.7 (4.6)	50.2 (4.0)	50.3 (4.3)	0.67	50.1 (3.7)	50.3 (4.7)	50.1 (4.0)	50.5 (4.3)	0.64	50.0 (4.3)	50.4 (4.0)	50.2 (4.2)	50.5 (4.3)	0.80
Age at 1st menstrual period (y)^a	13.1 (1.6)	12.9 (1.8)	13.2 (1.5)	12.8 (1.5)	0.007	13.1 (1.6)	12.9 (1.8)	13.0 (1.5)	12.9 (1.6)	0.37	12.9 (1.6)	13.1 (1.8)	12.9 (1.6)	13.0 (1.6)	0.47
Full term pregnancy^b															
Yes	245 (83.6)	143 (83.6)	322 (83.4)	657 (87.5)	0.17	239 (85.1)	134 (81.2)	298 (82.3)	593 (87.6)	0.06	239 (83.0)	134 (84.8)	348 (82.9)	543 (87.7)	0.11
Number of full term pregnancies^{*a}	2.1 (0.9)	2.4 (1.0)	2.3 (1.0)	2.4 (1.1)	<.001	2.1 (0.8)	2.4 (1.1)	2.3 (1.1)	2.5 (1.1)	<.001	2.1 (0.8)	2.4 (1.1)	2.4 (1.1)	2.4 (1.1)	<.001
Age at 1st full term pregnancy (y)^{*a}	25.5 (4.0)	25.2 (4.4)	25.7 (4.5)	24.7 (3.9)	<.001	25.4 (3.9)	25.4 (4.5)	25.4 (4.4)	25.0 (3.9)	0.37	25.4 (4.0)	25.3 (4.3)	25.3 (4.3)	25.0 (4.0)	0.58
Educational level^b					<.001					<.001					<.001
Primary/no schooling	95 (33.1)	102 (60.7)	98 (26.2)	407 (55.8)		95 (34.7)	97 (59.5)	110 (31.7)	369 (56.0)		99 (35.1)	93 (60.0)	139 (34.6)	340 (56.3)	
Technical/professional/secondary	132 (46.0)	43 (25.6)	167 (44.7)	245 (33.6)		115 (42.0)	47 (28.8)	135 (38.9)	219 (33.2)		118 (41.8)	44 (28.4)	165 (41.0)	189 (31.3)	
Longer education	60 (20.9)	23 (13.7)	109 (29.1)	78 (10.7)		64 (23.4)	19 (11.7)	102 (29.4)	71 (10.8)		65 (23.0)	18 (11.6)	98 (24.4)	75 (12.4)	
Physical activity^b					<.001					<.001					<.001
Inactive	58 (19.3)	59 (33.7)	68 (17.3)	251 (32.8)		55 (19.0)	62 (37.1)	76 (20.3)	239 (34.8)		57 (19.1)	60 (37.7)	99 (22.9)	216 (34.3)	
Moderately inactive	110 (36.7)	64 (36.6)	134 (34.0)	266 (34.8)		113 (39.0)	59 (35.3)	134 (35.8)	243 (35.4)		113 (37.9)	59 (37.1)	150 (34.7)	227 (36.1)	
Moderately active	73 (24.3)	32 (18.3)	118 (29.9)	145 (19.0)		69 (23.8)	23 (13.8)	84 (22.5)	117 (17.0)		67 (22.5)	25 (15.7)	95 (22.0)	106 (16.9)	
Active	55 (18.3)	18 (10.3)	64 (16.2)	84 (11.0)		48 (16.6)	23 (13.8)	68 (18.2)	76 (11.1)		56 (18.8)	15 (9.4)	78 (18.1)	66 (10.5)	
Missing	4 (1.3)	2 (1.1)	10 (2.5)	19 (2.5)		5 (1.7)	0 (0.0)	12 (3.2)	12 (1.7)		5 (1.7)	0 (0.0)	10 (2.3)	14 (2.2)	
Smoking status^b					<.001					<.001					<.001
Never	196 (65.3)	105 (60.0)	202 (51.3)	508 (66.4)		187 (64.5)	107 (64.1)	182 (48.7)	481 (70.0)		194 (65.1)	100 (62.9)	241 (55.8)	422 (67.1)	
Former smoker	50 (16.7)	36 (20.6)	109 (27.7)	145 (19.0)		49 (16.9)	34 (20.4)	119 (31.8)	114 (16.6)		55 (18.5)	28 (17.6)	122 (28.2)	111 (17.6)	
Current smoker	50 (16.7)	28 (16.0)	72 (18.3)	96 (12.5)		49 (16.9)	25 (15.0)	63 (16.8)	85 (12.4)		43 (14.4)	31 (19.5)	60 (13.9)	88 (14.0)	
Unknown	4 (1.3)	6 (3.4)	11 (2.8)	16 (2.1)		5 (1.7)	1 (0.6)	10 (2.7)	7 (1.0)		6 (2.0)	0 (0.0)	9 (2.1)	8 (1.3)	
Diabetes^b					<.001					<.001					<.001
Yes	5 (1.9)	5 (3.1)	5 (1.5)	41 (6.2)		5 (2.0)	5 (3.3)	3 (1.0)	42 (7.0)		4 (1.5)	6 (4.1)	4 (1.1)	41 (7.3)	
Alcohol intake (g/d)^{a,c}	8.0 (11.3)	7.1 (10.4)	8.6 (11.2)	5.6 (8.9)	<.001	8.1 (11.4)	7.7 (10.6)	8.7 (10.8)	6.0 (9.7)	<.001	7.5 (10.5)	8.8 (12.1)	7.5 (10.5)	6.6 (9.9)	0.10
Total energy intake (kcal/d)^a	2023.2 (566.6)	1965.9 (519.5)	1892.0 (535.1)	1866.2 (577.7)	<.001	2044.8 (555.4)	1963.9 (532.9)	1917.7 (527.7)	1897.0 (590.6)	0.002	2039.0 (554.2)	1970.7 (535.4)	1888.7 (500.2)	1915.1 (611.9)	0.002
C-peptide (ng/ml)^{a,k}	0.9 (0.3)	1.0 (0.3)	2.2 (1.2)	2.6 (1.4)	<.001	0.9 (0.3)	1.0 (0.3)	2.1 (1.0)	2.6 (1.5)	<.001	0.9 (0.3)	1.0 (0.3)	2.2 (1.1)	2.6 (1.5)	<.001
Height (cm)^a	161.8 (6.8)	159.1 (7.0)	163.5 (6.4)	159.5 (6.7)	<.001	160.9 (7.2)	160.1 (6.5)	161.9 (6.5)	159.5 (6.7)	<.001	161.5 (6.9)	158.9 (6.6)	161.7 (6.5)	159.4 (6.7)	<.001
Body Mass Index (kg/m²)^a	22.3 (1.7)	27.9 (2.7)	22.8 (1.5)	30.1 (4.4)	<.001	22.6 (2.1)	27.4 (3.2)	23.7 (2.3)	30.1 (4.7)	<.001	23.6 (3.0)	25.9 (3.6)	25.5 (4.1)	29.5 (5.0)	<.001
Waist circumference (cm)^a	73.0 (5.5)	85.4 (7.5)	75.4 (6.0)	90.7 (10.8)	<.001	72.2 (4.5)	86.7 (6.2)	74.1 (4.4)	92.2 (9.7)	<.001	73.8 (6.4)	84.4 (8.3)	77.2 (7.7)	91.7 (10.6)	<.000
Waist/Hip Ratio (cm/cm)^a	0.76 (0.06)	0.81 (0.07)	0.78 (0.06)	0.83 (0.07)	<.001	0.75 (0.05)	0.83 (0.07)	0.76 (0.05)	0.84 (0.06)	<.001	0.74 (0.03)	0.85 (0.06)	0.75 (0.03)	0.86 (0.05)	<.000

Note. ^aMean (SD). ^bN (%). ^cAmong parous women. NW=Normal weight. OW/OB=Overweight and obesity. ¹Metabolically healthy/normal weight (BMI < 25 kg/m² or Waist circumference <80 cm or Waist-to-hip ratio <0.8) plus below tertile 1 of C-peptide. ²Metabolically healthy/overweight (BMI ≥ 25 kg/m², or Waist circumference ≥80 cm or Waist-to-hip ratio ≥0.8), plus below tertile 1 of C-peptide. ³Metabolically unhealthy/normal weight (BMI < 25 kg/m² or Waist

circumference <80cm or Waist-to-hip ratio <0.8), plus above tertile 1 of C-peptide. ⁴Metabolically unhealthy/overweight (BMI ≥ 25 kg/m², or Waist circumference ≥ 80 cm or Waist-to-hip ratio ≥ 0.8), plus above tertile 1 of C-peptide. [^]Median (Interquartile range) among controls: 1.57 (1.05 – 2.32) and cases: 1.75 (1.16 – 2.64). [∞] Median (Interquartile range) among controls: 2.5 (0.3 – 10.8) and cases: 2.1 (0.2 – 9.3).

Table 3. Risk of endometrial cancer incidence associated with metabolic health-defined body size phenotypes using anthropometric and C-peptide tertile cut-points in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Body size definition	Metabolically healthy		Metabolically unhealthy		P
	Normal weight ¹	Overweight/Obesity ²	Normal weight ³	Overweight/Obesity ⁴	
BMI					
N cases/controls	121/179	81/94	166/228	449/316	
Basic model	1.00	1.34 (0.90-1.99) 0.45 (0.28-0.72)	1.06 (0.77-1.47)	2.29 (1.71-3.07) 1.00	<.0001 0.0008
Adjusted model	1.00	1.40 (0.91-2.15) 0.44 (0.26-0.74)	1.16 (0.82-1.64)	2.38 (1.73-3.27) 1.00	<.0001 0.0022
WC					
N cases/controls	110/180	83/84	169/205	397/290	
Basic model	1.00	1.86 (1.23-2.81) 0.69 (0.44-1.07)	1.41 (1.02-1.95)	2.58 (1.89-3.53) 1.00	<.0001 0.0975
Adjusted model	1.00	1.94 (1.24-3.04) 0.80 (0.49-1.31)	1.48 (1.05-2.10)	2.69 (1.92-3.77) 1.00	<.0001 0.3821
WHR					
N cases/controls	125/173	68/91	225/207	341/288	
Basic model	1.00	1.06 (0.71-1.60) 0.46 (0.28-0.76)	1.55 (1.14-2.11)	1.76 (1.30-2.39) 1.00	<.0001 0.0025
Adjusted model	1.00	1.17 (0.75-1.81) 0.43 (0.25-0.76)	1.68 (1.21-2.35)	1.83 (1.32-2.54) 1.00	<.0001 0.0033

Note. In bold we highlight the results that were statistically significant. Sub-sample analyses are also presented in this table. Values are OR (95% CI). BMI=Body Mass Index. WC=Waist Circumference. WHR=Waist-to-Hip ratio. Basic model was conditioned on matching factors only. Adjusted model was conditioned on matching factors, with additional adjustment for age at menopause, age at menarche, parity, hormone use, physical activity index, smoking status, educational level, alcohol intake, height, energy intake and diabetes. P-value for trend. ¹Metabolically healthy/normal weight (BMI < 25 kg/m² or Waist circumference <80 cm or Waist-to-hip ratio <0.8) plus below tertile 1 of C-peptide. ²Metabolically healthy/overweight (BMI ≥ 25 kg/m², or Waist circumference ≥80 cm or Waist-to-hip ratio ≥0.8), plus below tertile 1 of C-peptide. ³Metabolically unhealthy/normal weight (BMI < 25 kg/m² or Waist circumference <80cm or Waist-to-hip ratio <0.8), plus above tertile 1 of C-peptide. ⁴Metabolically unhealthy/overweight (BMI ≥ 25 kg/m², or Waist circumference ≥80cm or Waist-to-hip ratio ≥0.8), plus above tertile 1 of C-peptide.