Serum levels of C-peptide, IGFBP-1 and IGFBP-2 and endometrial cancer risk; results from the European Prospective Investigation into Cancer and Nutrition

Anne E. Cust[1,2,3], Naomi E. Allen[4], Sabina Rinaldi[1], Laure Dossus[1,5], Christine Friedenreich[6], Anja Olsen[7], Anne Tjønneland[7], Kim Overvad[8], Françoise Clavel-Chapelon[9], Marie-Christine Boutron-Ruault[9], Jakob Linseisen[5], Jenny Chang-Claude[5], Heiner Boeing[10], Mandy Schulz[10], Vassiliki Benetou[11], Antonia Trichopoulou[11], Dimitrios Trichopoulos[11], Domenico Palli[12], Franco Berrino[13], Rosario Tumino[14], Amalia Mattiello[15], Paolo Vineis[16], J. Ramón Quirós[17], Antonio Agudo[18], Maria-Jose Sánchez[19], Nerea Larrañaga[20], Carmen Navarro[21], Eva Ardanaz[22], H. Bas Bueno-de-Mesquita[23], Petra H.M. Peeters[24], Carla H. van Gils[24], Sheila Bingham[25,26], Kay-Tee Khaw[27], Tim Key[4], Nadia Slimani[1], Elio Riboli[16] and Rudolf Kaaks[1,5]*

[1]*Nutrition and Hormones Unit, International Agency for Research on Cancer, Lyon, France*
[2]*School of Public Health, University of Sydney, Australia*
[3]*Center for Research in Human Nutrition Rhône-Alpes, Université Lyon 1, France*
[4]*Cancer Research UK Epidemiology Unit, University of Oxford, United Kingdom*
[5]*Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
[6]*Division of Population Health and Information, Alberta Cancer Board, Calgary, Alberta, Canada*
[7]*Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark*
[8]*Department of Clinical Epidemiology, Aarhus University Hospital, Denmark*
[9]*INSERM ERI20, Paris-Sud University EA4045, and Institut Gustave Roussy, Villejuif, France*
[10]*Department of Hygiene and Epidemiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany*
[11]*University of Athens Medical School, Athens, Greece*
[12]*Molecular and Nutritional Epidemiology Unit, CSPO-Scientific Institute of Tuscany, Florence, Italy*
[13]*Epidemiology Unit, National Cancer Institute, Milan, Italy*
[14]*Cancer Registry, Azienda Ospedaliera "Civile M.P. Arezzo", Ragusa, Italy*
[15]*Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy*
[16]*Department of Epidemiology and Public Health, Imperial College London, United Kingdom*
[17]*Public Health and Health Planning Directorate, Asturias, Spain*
[18]*Department of Epidemiology, IDIBELL, Catalan Institute of Oncology, Barcelona, Spain*
[19]*Andalusian School of Public Health, Granada, Spain*
[20]*Public Health Department of Gipuzkoa, Basque Government, San Sebastian, Spain*
[21]*Epidemiology Department, Murcia Health Council, Spain*
[22]*Public Health Institute of Navarra, Pamplona, Spain*
[23]*Center for Nutrition and Health, National Institute of Public Health and the Environment, Bilthoven, The Netherlands*
[24]*Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands*
[25]*MRC Dunn Human Nutrition Unit, Cambridge, United Kingdom*
[26]*MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, Department of Public Health and Primary Care, University of Cambridge, United Kingdom*
[27]*Clinical Gerontology, Department of Public Health and Primary Care, University of Cambridge, United Kingdom*

We conducted a case-control study nested within the European Prospective Investigation into Cancer and Nutrition, to examine the associations between prediagnostic serum concentrations of C-peptide, insulin-like growth factor binding protein (IGFBP)-1 and IGFBP-2, and endometrial cancer risk. Among pre- and postmenopausal women, who were not currently using exogenous hormones, 286 women developed incident endometrial cancer during an average 5.1 years follow-up. Using risk set sampling, 555 matched control subjects were selected. In conditional logistic regression models adjusted for matching factors only, endometrial cancer risk increased with increasing serum levels of C-peptide (relative risks (RR) for the top *vs.* bottom quartile = 2.13 [95% confidence interval (CI) 1.33–3.41], p_{trend} = 0.001, and decreasing serum levels of IGFBP-2 (RR for the top *vs.* bottom quartile = 0.56 [95% CI 0.35–0.90], p_{trend} = 0.03, but was not significantly associated with IGFBP-1 levels (RR for the top *vs.* bottom quartile = 0.76 [95% CI 0.47–1.21], p_{trend} = 0.25). In BMI-adjusted models, only the C-peptide association remained marginally statistically significant (RR for the top *vs.* bottom quartile = 1.56 [95% CI 0.94–2.57], p_{trend} = 0.05 for C-peptide; 0.84 [95% CI 0.50–1.40], p_{trend} = 0.74 for IGFBP-2; and 1.08 [95% CI 0.65–1.78], p_{trend} = 0.86 for IGFBP-1 levels). These associations were stronger among nonfasting women (≤6 hr since last meal; 63% of subjects) but were not evident among fasting women, although the interactions were not statistically significant. The C-peptide-risk association was substantially attenuated after adjustment for free estradiol in postmenopausal women (RR for the top *vs.* bottom quartile = 1.28 [95% CI 0.67–2.45], p_{trend} = 0.42. Our results provide modest sup-

Abbreviations: BMI, body mass index; CI, confidence interval; DHEAS, dehydroepiandrosterone sulphate; EPIC, European Prospective Investigation into Cancer and Nutrition; HRT, hormone replacement therapy; IARC, International Agency for Research on Cancer; IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; MET, metabolic equivalent values; OC, oral contraceptives; RR, relative risk; SHBG, serum sex hormone binding globulin; WHR, waist-hip ratio.

Grant sponsors: "Europe Against Cancer" Programme of the European Commission (SANCO), Ligue contre le Cancer (France), Société 3M (France), Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM), German Cancer Aid, German Cancer Research Centre, German Federal Ministry of Education and Research, Danish Cancer Society, Health Research Fund (FIS) of the Spanish Ministry of Health, Spanish Regional Governments of Andalusia, Asturias, Basque Country, Murcia and Navarra, ant the Catalan Institute of Oncology, Cancer Research UK, Medical Research Council (UK), Stroke Association (UK), British Heart Foundation, Department of Health (UK), Food Standards Agency (UK), Wellcome Trust (UK), Greek Ministry of Health, Greek Ministry of Education, Italian Association for Research on Cancer (AIRC), Italian National Research Council, Dutch Ministry of Public Health, Welfare and Sports, Dutch Ministry of Health, Dutch Prevention Funds, LK Research Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Swedish Cancer Society, Swedish Scientific Council, Regional Government of Skane (Sweden), Norwegian Cancer Society; Grant sponsor: ISCIII Network RCESP; Grant number: C03/09; Grant sponsor: RETICC; Grant number: C03/10.

***Correspondence to:** Division of Cancer Epidemiology, German National Cancer Center (DKFZ), 69120 Heidelberg, Germany.
Fax: +49-6221-4222-03. E-mail: r.kaaks@dkfz.de

port to the hypothesis that hyperinsulinaemia is a risk factor for endometrial cancer.

Key words: endometrial cancer; insulin; IGFBP-1; IGFBP-2; C-peptide; prospective

In 2002, endometrial cancer was diagnosed in ~200,000 women worldwide and 50,000 women died of the disease.[1] It is the 4th most common cancer in women in western countries, where age-standardized incidence rates are 4.5 times higher than in less developed regions.[1] Variations in incidence rates by region, over time, or following migration from low-risk to high-risk countries support data from epidemiological studies that have shown lifestyle factors to be important predictors of endometrial cancer risk.[2]

Whilst exposure to unopposed estrogens is strongly implicated in the aetiology of endometrial cancer,[2] other hormonal factors, such as those relating to insulin resistance, may also play a role in the development of this disease. Indeed, excess weight and to a lesser extent physical inactivity, both of which are key determinants of insulin resistance,[3,4] are estimated to cause at least half of all endometrial cancer cases in Europe and the USA[3,5] It has been hypothesized that insulin resistance and the resulting compensatory chronic hyperinsulinaemia could be an important mediator of their effects on endometrial cancer risk.[2,4] In a recent prospective case-control study nested within 3 cohorts in New York, northern Sweden and Milan,[6] high blood levels of C-peptide, a biomarker of pancreatic insulin production, were associated with up to a 4-fold increase in endometrial cancer risk, even after adjusting for body mass index (BMI; kg/m^2). Two other case-control studies in the USA[7] and Finland[8] also showed an increased risk with increasing circulating C-peptide and insulin levels, respectively. Furthermore, Type II diabetes, which is generally associated with hyperinsulinaemia for many years both before and after disease onset, is a recognized risk factor for endometrial cancer.[9,10]

Several biological mechanisms have been proposed implicating hyperinsulinaemia in the aetiology of endometrial cancer[2,4]; insulin itself may act directly on endometrial tissue as a mitogenic and anti-apoptotic growth factor, or insulin may act indirectly by increasing circulating levels of free estrogens and androgens through down-regulation of sex hormone binding globulin (SHBG) and up-regulation of ovarian sex steroid production.[2,4] Insulin may also increase IGF-I bioactivity by reducing the circulating and endometrial tissue levels of insulin-like growth factor binding proteins (IGFBP)-1 and IGFBP-2,[11–14] although epidemiological evidence of a possible association of endometrial cancer risk with serum levels of IGFBP-1[6,8,15–18] and IGFBP-2[6,8] has so far been inconclusive.

To clarify the role of prediagnostic serum C-peptide, IGFBP-1 and IGFBP-2 levels as possible risk factors for endometrial cancer, we conducted a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) prospective cohort. We also examined to what extent the associations between these peptide hormone levels and endometrial cancer risk are explained by differences in obesity, physical inactivity and other traditional risk factors, and whether hyperinsulinaemia influences risk *via* changes in endogenous sex hormone metabolism. IGF-1 levels were not measured in our study because circulating IGF-I levels may not be a good marker of the local expression and action of IGF-I in the endometrium,[6] and because IGF-1 has a complex, non-linear relationship with BMI and energy balance.[19] This is the largest prospective study of its kind to date, comprising 286 incident cases of endometrial cancer in pre- and post-menopausal women, and 555 matched controls.

Material and methods

Study population

EPIC recruitment procedures and collection of questionnaire data, anthropometric measurements and blood samples have been previously described in detail.[20] In brief, extensive standardized questionnaire data were collected between 1992 and 1998, from ~366,500 women and 153,500 men in 10 western European countries, of whom about 386,000 also provided a blood sample. The present study includes endometrial cancer cases occurring after blood collection, and matched control subjects, from 8 of the 10 participating countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Spain and the United Kingdom. Norway was not included in the present study because blood samples had been collected only recently on a subsample of cohort participants, and only very few cases of endometrial cancer had been diagnosed when the present study was initiated. Sweden was not included because a separate study within their cohort had already been planned.

Data collection

Data on nondietary lifestyle and health factors were collected *via* a questionnaire that included menstrual and reproductive history, current and previous use of oral contraceptives (OC) and postmenopausal hormone replacement therapy (HRT), history of previous illness and disorders or surgical operations, lifetime history of tobacco smoking and alcohol consumption, past-year physical activity, level of education and brief occupational history. Height, weight and waist and hip circumferences were measured according to standardized protocols, in light dressing, except for part of the Oxford cohort, where height and weight were self-reported. A summary measure of physical activity, combining recreational and household activity in MET-hr/week, and the definition of menopausal status, have been previously described in detail.[21,22] Type of occupational physical activity was measured in the EPIC questionnaire but could not be transformed into a continuous variable; nevertheless, results of statistical analyses were similar when we substituted the continuous summary variable with a categorical variable that included occupational, household and recreational activity. All baseline questionnaire and follow-up data were entered into a central database at the International Agency for Research on Cancer (IARC) in Lyon, France.

Collection and storage of blood samples

Blood samples were collected according to a standardized protocol. From each subject, 30 ml of blood was drawn and stored at 5–10°C, protected from light, and transferred to local laboratories for further processing and aliquoting. An exception was the EPIC-Oxford centre (UK), where blood samples were collected through a network of general practitioners and transported to a central laboratory by mail at ambient temperature. The syringes for the preparation of blood plasma contained trisodium citrate as anticoagulant. After centrifugation, blood fractions were aliquoted into 28 plastic straws of 0.5 ml each and stored at −196°C. In Denmark, blood fractions were aliquoted into 1-ml tubes and stored at −150°C. The time at last consumption of food or drink was recorded, and used to define samples as 'fasting' (>6 hr since last food or drink) or 'nonfasting' (≤6 hr); a 6-hr cut-off was chosen because plasma insulin concentrations usually return to baseline levels within this time,[23,24] and this was also a natural cut-off point within our study distribution (Fig. 1). The majority of women in this analysis had nonfasting samples (63%; n = 181 cases, 347 matched controls), 32% had fasting samples (n = 90 cases, 179 matched controls) and fasting status was unknown for 5% (n = 15 cases, 29 matched controls).

Follow-up for cancer incidence and vital status

In Denmark, Italy, the Netherlands, the United Kingdom and Spain, incident cancer cases were identified through record linkage with regional cancer registries. In Germany, France and Greece, follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. Data on vital

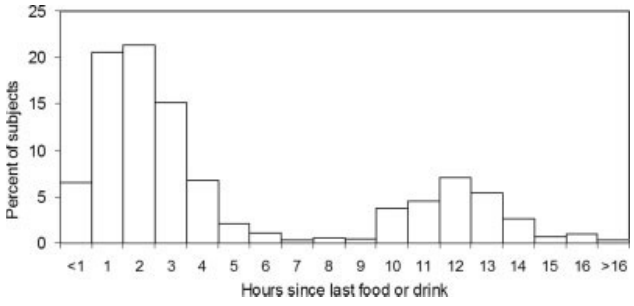

FIGURE 1 – The distribution of subjects according to the number of hours since last food or drink at the time of blood donation.

status in most EPIC study centres were collected from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). Vital status was known for 98.4% of all EPIC participants as of April 2004. For each EPIC study centre, closure dates of the study period were defined as the date of the last complete follow-up for both cancer incidence and vital status (dates varied between June 1999 and December 2003 between centres). The average duration of follow-up for this analysis was 5.1 years.

Selection of case and control subjects

Case subjects were women who were diagnosed with endometrial cancer after the initial blood donation and before the end of the study period in each study centre. Exclusion criteria for cases and controls for our study included women who, at the time of blood donation, were using exogenous hormones (OCs or HRT), or had a previous diagnosis of cancer (except nonmelanoma skin cancer), or had had a hysterectomy. We further excluded endometrial tumours of known nonepithelial or nonmalignant morphology.[25] A total of 135,953 women met these eligibility criteria, of whom 300 women were diagnosed with endometrial cancer during the follow-up period. After further exclusion of 14 cases with insufficient serum in the biological bank, matched control subjects were identified for 286 cases. In each country, the number of cases and the average duration of follow-up in years, respectively, was Denmark: 67, 5.0; Italy 61, 5.0; Spain 42, 5.4; Netherlands 36, 5.4; UK 34, 4.7; Germany 20, 4.5; France 17, 7.6 and Greece 9, 3.0. The cancer diagnosis was confirmed by histology (92%), clinical examination (7%) or self-report, tomography scan, surgery or autopsy (1%). Detailed tumour morphology was specified for 143 cases (50%), of which 134 cases (94%) were classified as Type I (generally estrogen-dependent endometrioid adenocarcinomas) and 9 cases (6%) as Type II (mostly nonestrogen-dependent, serous papillary, clear cell or squamous adenocarcinomas).[25,26]

For each case subject, up to 2 control subjects were chosen at random from all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. An incidence density sampling protocol for control selection was used, such that controls could include subjects who became a case later in time, while each control could also be sampled more than once. Matching criteria included: study centre, menopausal status (premenopausal, postmenopausal, unknown), age at blood collection ($\pm$6 months), time of the day of blood collection ($\pm$1 hr), time between blood draw and last consumption of foods or drinks (<3, 3–6, >6 hr, unknown), and for premenopausal women, phase of menstrual cycle.[27] Two matched controls were identified for 269 cases, and 1 matched control for 17 cases (total of 555 controls).

All participants gave written consent for future analyses of their blood samples, and the study was approved by the local ethics committees in the participating countries and the Internal Review Board of the IARC.

Hormone assays

All hormone assays were performed at the laboratory of the Hormones and Cancer Group at the IARC, Lyon, France, by laboratory staff blinded to the case-control status of the study subjects. The serum samples of each matched case-control set were analyzed with sets from the same centre, within the same batch (*i.e.*, using the same immunoassay kit) on the same day.

IGFBP-2 concentrations were measured by radioimmunoassay, and IGFBP-1 by immunoradiometric assays, from Diagnostic System Laboratories (Webster, TX). C-peptide concentrations were measured by immunoradiometric assay by Immunotech (Marseille, France). Three additional sera were inserted randomly in each batch as a quality control measure. The intrabatch coefficients of variation (CV) were 2.6% for a C-peptide concentration of 0.50 nmol/l, 4.6% for an IGFBP-1 concentration of 1.86 nmol/l, and 4.7% for an IGFBP-2 concentration of 25.6 nmol/l. Interbatch CVs were 11.3% for C-peptide, 24.2% for IGFBP-1 and 11.0% for IGFBP-2. Some measurements were unable to be obtained for C-peptide (1 case), IGFBP-1 (1 case) and IGFBP-2 (4 cases, 10 controls).

Endogenous sex steroid hormone levels (testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), estrone, estradiol, SHBG) were measured, using reproducible and valid techniques, as described in detail elsewhere,[28] and are to be examined in a separate paper. Levels of circulating estradiol were only measured in postmenopausal women because, in premenopausal women, chronic hyperinsulinaemia has little effect on estrogen levels, which are regulated through negative feedback of estradiol on the pituitary secretion of FSH, and because estradiol levels vary markedly through a woman's menstrual cycle, thus making interindividual differences difficult to measure accurately.[2] Serum concentrations of free testosterone and free estradiol, unbound to SHBG or albumin, were calculated from the absolute concentrations of each of the steroids and SHBG, using a method that has been validated in postmenopausal women.[29]

Statistical analyses

Circulating levels of peptide and sex steroid hormones were log-transformed to normalize their distributions. Differences in baseline characteristics and the geometric mean peptide levels between cases and controls (using the average of the 2 matched controls) were compared, using the paired *t*-test for continuous variables and the chi-square test for categorical variables. Pearson's partial correlation coefficients, adjusted for age at blood collection, laboratory batch and case-control status, were calculated to assess the correlations between levels of C-peptide, IGFBP-1 and IGFBP-2, anthropometric measures, physical activity and endogenous sex steroid hormone levels. Analysis of variance was used to examine the contribution of age, laboratory batch, country, fasting status, menopausal status, BMI and physical activity level on the between-subject variation in peptide levels.

Conditional logistic regression models were used to estimate relative risks (RR) and 95% confidence intervals (CI) for the associations between serum peptide levels and endometrial cancer risk. The prospective design and use of incidence density sampling for selecting controls in our study permitted the direct estimation of the RR rather than the odds ratio.[30] Quartile cut-points for C-peptide, IGFBP-1 and IGFBP-2 were based on the distribution of each variable in control subjects only and for all centres combined. Likelihood ratio tests using medians for each quartile category were used to assess linear trends in RR.

Multivariate logistic regression was used to examine the effects of potential confounders other than those controlled for by matching, including BMI (kg/m^2; as a continuous variable), waist circumference (cm; quartiles: <76, 76–82, 83–91, $\geq$92), waist-hip ratio (quartiles: <0.76, 0.76–0.80, 0.81–0.84, $\geq$0.85), age at menarche (<12, 12, 13, 14, $\geq$15, missing), age at menopause (<44, 44–47, 48–50, 51–52, 53–54, $\geq$55, missing), parity (nulliparous, 1 or more, missing), age at birth of last child (nulliparous, <25, 25–29, 30–34, $\geq$35, missing), OC use (never, past, missing),

TABLE I – BASELINE DEMOGRAPHIC AND LIFESTYLE CHARACTERISTICS FOR WOMEN WHO DEVELOPED ENDOMETRIAL CANCER (CASES) AND CONTROL SUBJECTS

	Cases	Controls	p-value[1]
Total number of subjects	286	555	
Menopausal status			
Premenopausal (n, 19%)	55	107	
Postmenopausal (n, 67%)	192	374	
Unknown (n, 13%)	39	74	
Age at blood collection	56.9 (45.4–67.9)	56.9 (45.0–68.3)	
Age at diagnosis	59.9 (47.0–71.0)	–	
Years between blood collection and diagnosis	3.0 (0.0–6.0)	–	
Anthropometric measures			
Height (cm)	160.4 (149.7–171.0)	160.5 (149.5–171.7)	0.77
Weight (kg)	72.1 (53.5–97.9)	68.0 (51.0–88.6)	<0.0001
BMI (kg/m^2)	28.1 (20.9–37.9)	26.5 (20.2–34.8)	<0.0001
Waist (cm)	86.6 (67.2–112.0)	83.0 (67.5–104.0)	<0.0001
Hip (cm)	105.7(90.3–126.0)	102.6 (89.5–120.0)	<0.0001
WHR	0.82 (0.72–0.93)	0.81 (0.70–0.93)	0.05
Obese (% BMI $\geq$ 30 kg/m^2)	32.5	18.2	<0.0001
Age at menarche	13.1 (11.0–15.0)	13.3 (11.0–16.0)	0.10
Age at first pregnancy	24.5 (18.0–31.0)	25.1 (19.0–34.0)	0.18
Age at birth of last child	29.3 (21.0–37.0)	30.3 (23.0–38.0)	0.02
Nulliparous (%)	19.0	9.9	0.0002
Number of pregnancies in parous women	2.3 (1.0–4.0)	2.4 (1.0–5.0)	0.27
Age at menopause	51.2 (45.0–56.0)	49.7 (41.0–55.0)	0.0004
Previous oral contraceptive use (%)	33.7	42.9	0.01
Previous hormone replacement therapy use (%)	20.3	15.0	0.06
Self-reported diabetes (%)	3.6	3.9	0.80
Completed secondary school or equivalent (%)	60.4	54.9	0.14
Smoking status (%)			
Never smoker	66.3	59.9	0.19
Ex smoker	18.3	22.0	
Current smoker	15.4	18.0	
Household and recreational physical activity[2]	97.6 (24.5–190.6)	106.3 (30.0–198.6)	0.01

BMI, body mass index; WHR, waist–hip ratio. Data are presented as means (5th–95th percentiles) or percentages. Missing values are excluded from calculations.
[1]Calculated using paired t-tests for continuous variables and chi-square tests for categorical variables.–[2]Calculated as MET-hr/week.

HRT use (never, past, missing), hypertension (yes, no, missing), diabetes (yes, no, missing), smoking (never, past, current, missing), physical activity level (MET-hours/week in quartiles: <61.5, 61.5–96.9, 97.0–141.9, ≥142.0) and education (completed secondary school: yes, no, missing). Other than measures of adiposity, none of these variables appreciably altered the RR of the associations between peptide levels and endometrial cancer risk, and their inclusion decreased the precision of the risk estimates, therefore we present only the 'crude' (adjustment for matching variables only) and BMI-adjusted values in this article.

In addition, we examined whether serum sex hormone levels potentially mediated the association between hyperinsulinaemia and endometrial cancer risk by including free estradiol (in postmenopausal women), SHBG and free testosterone as continuous variables in the crude regression model.

Chi-square tests were used to examine heterogeneity of endometrial cancer risk estimates associated with a doubling of C-peptide, IGFBP-1 or IGFBP-2 levels among subgroups. The subgroups examined included country, menopausal status, age at cancer diagnosis (<55, ≥55 years), previous HRT use, BMI, fasting status (≤6 hr, >6 hr since last meal), physical activity level, smoking status and lag time between blood donation and diagnosis (<2 years, ≥2 years). All statistical tests were 2-tailed, and p-values < 0.05 were considered statistically significant. All statistical analyses were performed using the SAS software package, version 9.1 (SAS Institute, Cary, NC).

Results

Baseline characteristics of the study participants are shown in Table I. Compared to control subjects, endometrial cancer cases were more likely to be obese, nulliparous, finish child-bearing at a younger age, and have a later age at menopause. Cases also more often reported a lower physical activity level (MET-hours/week), and to have never used OCs. There were no significant differences between cases and controls in height, age at menarche, age at first full-term pregnancy, number of pregnancies, prevalence of self-reported diabetes, past or current smoking habits, or previous use of HRT. There were greater differences in measures of adiposity between cases and controls among postmenopausal women (% obese = 34.9% *vs.* 19.8%, respectively, $p < 0.0001$) than among premenopausal women (% obese = 23.6% *vs.* 15.0%, respectively, $p = 0.17$). The distribution of subjects by fasting status is shown in Figure 1.

Serum C-peptide levels were positively correlated with measures of adiposity in both fasting and nonfasting samples, as determined by Pearson's partial correlation coefficients adjusted for age at blood collection, laboratory batch and case-control status (Table II). This correlation was observed most strongly with BMI and waist circumference, and to a lesser extent with waist-hip ratio. In addition, C-peptide was positively correlated with free estradiol (postmenopausal women) and free testosterone, and negatively correlated with IGFBP-1, IGFBP-2 and SHBG. Correlations were generally weaker in nonfasting samples than in fasting samples (Table II), but did not differ according to menopausal status or physical activity level (data not shown). Serum levels of C-peptide, IGFBP-1 or IGFBP-2 were not correlated with androstenedione, DHEAS (data not shown) or questionnaire measurement of physical activity.

After adjustment for age, laboratory batch and country, the percentage of between-subject variation accounted for by fasting status was 11% for C-peptide levels, 5% for IGFBP-1 levels, and 2% for IGFBP-2 levels; BMI accounted for 16, 13 and 17% of the variation in C-peptide, IGFBP-1 and IGFBP-2 levels, respectively. Menopausal status, physical activity and age predicted less than

TABLE II – PEARSON PARTIAL CORRELATION COEFFICIENTS[1] AND 95% CONFIDENCE INTERVALS BETWEEN SERUM LEVELS OF C-PEPTIDE, IGFBP-1 AND IGFBP-2, SEX HORMONES AND ANTHROPOMETRIC MEASURES, IN FASTING AND NON-FASTING SAMPLES

	C-peptide		IGFBP-1		IGFBP-2	
	Fasting	Nonfasting	Fasting	Nonfasting	Fasting	Nonfasting
IGFBP-1	−0.62 (−0.69, −0.54)	−0.43 (−0.50, −0.36)		0.45 (0.38, 0.52)		
IGFBP-2	−0.58 (−0.65, −0.49)	−0.36 (−0.43, −0.28)	0.60 (0.51, 0.67)	0.45 (0.38, 0.52)		
BMI	0.53 (0.43, 0.61)	0.33 (0.26, 0.41)	−0.43 (−0.53, −0.33)	−0.35 (−0.43, −0.28)	−0.46 (−0.55, −0.36)	−0.43 (−0.50, −0.35)
Waist circumference	0.44 (0.34, 0.53)	0.40 (0.32, 0.47)	−0.37 (−0.47, −0.26)	−0.39 (−0.46, −0.31)	−0.45 (−0.54, −0.34)	−0.47 (−0.54, −0.40)
WHR	0.24 (0.13, 0.35)	0.30 (0.22, 0.37)	−0.22 (−0.33, −0.10)	−0.31 (−0.38, −0.23)	−0.30 (−0.41, −0.19)	−0.40 (−0.47, −0.33)
Physical activity	0.11 (−0.02, 0.22)	−0.07 (−0.15, 0.02)	−0.08 (−0.20, 0.04)	0.08 (0.00, 0.17)	−0.09 (−0.21, 0.03)	0.06 (−0.02, 0.15)
SHBG	−0.49 (−0.58, −0.40)	−0.41 (−0.48, −0.34)	0.49 (0.39, 0.58)	0.49 (0.42, 0.55)	0.58 (0.49, 0.65)	0.65 (0.60, 0.70)
Testosterone	0.13 (0.01, 0.25)	−0.02 (−0.11, 0.07)	−0.16 (−0.28, −0.04)	−0.06 (−0.14, 0.03)	−0.16 (−0.28, −0.04)	−0.10 (−0.19, −0.02)
Free testosterone	0.36 (0.24, 0.46)	0.18 (0.10, 0.27)	−0.39 (−0.49, −0.28)	−0.29 (−0.37, −0.21)	−0.44 (−0.54, −0.33)	−0.41 (−0.48, −0.33)
Estradiol[2]	0.22 (0.07, 0.36)	0.13 (0.02, 0.23)	−0.19 (−0.33, −0.04)	−0.13 (−0.23, −0.03)	−0.36 (−0.48, −0.22)	−0.28 (−0.37, −0.18)
Free estradiol[2]	0.39 (0.25, 0.51)	0.27 (0.17, 0.37)	−0.34 (−0.47, −0.20)	−0.29 (−0.38, −0.19)	−0.54 (−0.64, −0.42)	−0.50 (−0.58, −0.42)

BMI, body mass index; WHR, waist–hip ratio.
[1]Pearson partial correlation coefficients were adjusted for age, case–control status and laboratory batch.–[2]Serum estradiol concentration was measured in postmenopausal women only because of its considerable intraindividual variation during the menstrual cycle among premenopausal women.

TABLE III – GEOMETRIC MEANS (95% CONFIDENCE INTERVALS) OF C-PEPTIDE, IGFBP-1 AND IGFBP-2 FOR WOMEN WHO DEVELOPED ENDOMETRIAL CANCER (CASES) AND CONTROL SUBJECTS

	Cases	Controls	*p*-value[1]
All women[2]			
C-peptide	0.87 (0.82–0.93)	0.81 (0.77–0.86)	0.008
IGFBP-1	0.55 (0.48–0.62)	0.56 (0.50–0.62)	0.63
IGFBP-2	10.40 (9.50–11.30)	11.00 (10.20–11.80)	0.09
Fasting women[3]			
C-peptide	0.65 (0.61–0.70)	0.65 (0.62–0.68)	0.78
IGFBP-1	0.94 (0.83–1.08)	0.88 (0.80–0.96)	0.38
IGFBP-2	9.59 (8.46–10.90)	9.37 (8.58–10.20)	0.69
Nonfasting women[3]			
C-peptide	0.97 (0.90–1.04)	0.87 (0.82–0.92)	0.01
IGFBP-1	0.40 (0.35–0.46)	0.42 (0.38–0.46)	0.50
IGFBP-2	12.30 (11.30–13.50)	13.70 (12.80–14.60)	0.02

Concentrations are expressed as nmol/l.
[1]Calculated using paired *t*-tests.–[2]Adjusted for age, BMI and fasting status.–[3]Adjusted for age and BMI.

TABLE IV – RELATIVE RISK ESTIMATES FOR ENDOMETRIAL CANCER RISK ACCORDING TO SERUM LEVELS OF C-PEPTIDE, IGFBP-1 AND IGFBP-2, IN ALL WOMEN

Peptide hormone Quartile[1] cut-points (nmol/l)	No. of cases/ controls	Crude[2] RRs and 95% CIs	BMI-adjusted[2] RRs and 95% CIs
C-peptide			
<0.56	55/138	1.00	1.00
0.56–0.75	59/139	1.10 (0.70–1.73)	0.98 (0.62–1.55)
0.76–1.09	76/138	1.57 (1.00–2.46)	1.25 (0.78–2.00)
>1.09	95/138	2.13 (1.33–3.41)	1.56 (0.94–2.57)
p_{trend}		0.001	0.05
IGFBP-1			
<0.30	84/139	1.00	1.00
0.30–0.57	68/138	0.76 (0.50–1.16)	0.91 (0.59–1.40)
0.58–1.07	64/138	0.71 (0.45–1.11)	0.85 (0.54–1.35)
>1.07	69/138	0.76 (0.47–1.21)	1.08 (0.65–1.78)
p_{trend}		0.25	0.86
IGFBP-2			
<8.02	81/135	1.00	1.00
8.02–12.46	73/133	0.92 (0.61–1.38)	1.07 (0.70–1.63)
12.47–19.68	78/134	0.95 (0.62–1.46)	1.27 (0.80–2.00)
>19.68	50/135	0.56 (0.35–0.90)	0.84 (0.50–1.40)
p_{trend}		0.03	0.74

[1]Quartile cut-points for C-peptide, IGFBP-1 and IGFBP-2 were based on the distribution of each variable in control subjects only and for all centres combined.–[2]Conditional logistic regression models were adjusted for matching factors (study centre, menopausal status, age at blood collection, time of day of blood collection, fasting status and in premenopausal women—phase of menstrual cycle).

1% of the between-subject variation in levels. In nonfasting samples, the geometric mean serum C-peptide concentration was higher and the mean IGFBP-1 concentration was lower than in fasting samples ($p < 0.0001$ for both) after adjustment for age, case-control status and country, but the mean IGFBP-2 concentration was not significantly different by fasting status ($p = 0.17$). After adjustment for age, BMI and fasting status in all women, cases had a statistically significantly 7% higher mean serum C-peptide concentration, and nonstatistically significant lower IGFBP-1 and IGFBP-2 mean concentrations, compared to controls (Table III). The differences were not apparent among fasting women (Table III).

In logistic regression analyses, there were increases in endometrial cancer risk with increasing serum levels of C-peptide (RR for the top *vs.* bottom quartile = 2.13 [95% CI 1.33–3.41], $p_{trend} = 0.001$), and decreasing levels of IGFBP-2 (RR for the top *vs.* bottom quartile = 0.56 [95% CI 0.35–0.90], $p_{trend} = 0.03$). However, IGFBP-1 levels were not significantly associated with risk (RR for the top *vs.* bottom quartile = 0.76 [95% CI 0.47–1.21], $p_{trend} = 0.25$) (Table IV). Adjustment for BMI attenuated

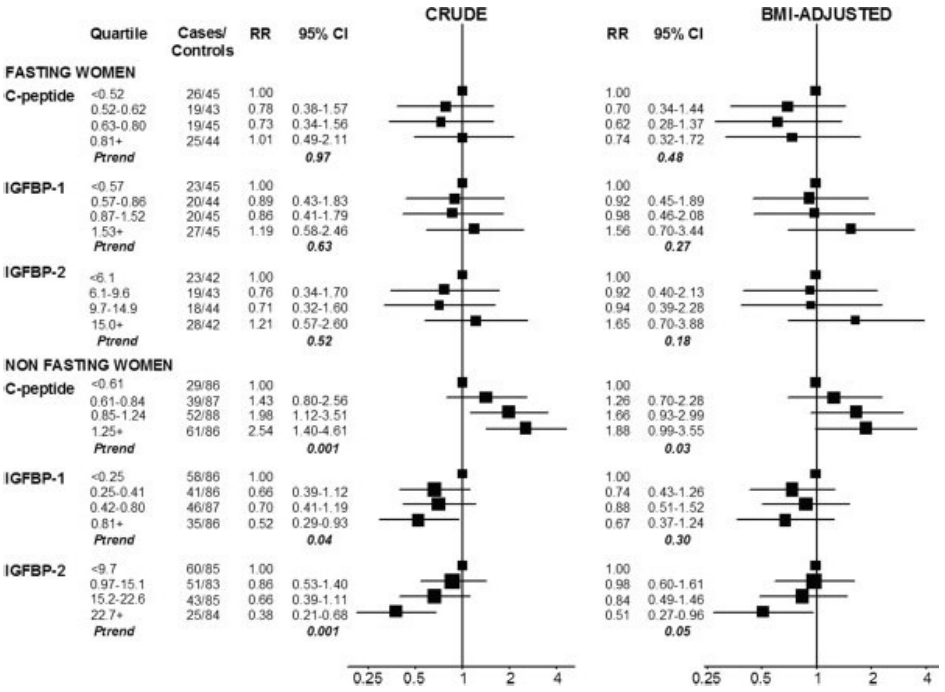

FIGURE 2 – RR estimates and 95% CI for endometrial cancer risk according to quartiles of serum levels of C-peptide, IGFBP-1 and IGFBP-2, in fasting and nonfasting women. Solid squares = RR. The area of the square reflects the centre-specific statistical weight (inverse of the variance). Horizontal bars = 95% CIs. p_{trend} = p value for a test of linear trend (two-sided test), using medians for each quartile category. Excludes 15 cases and 29 matched controls that have an unknown fasting status. Conditional logistic regression models, adjusted for matching factors (study centre, menopausal status, age, time of the day of blood collection, fasting status, phase of menstrual cycle) were used to calculate RRs and 95% CIs. Quartile cut-points for C-peptide, IGFBP-1 and IGFBP-2 were based on the distribution of each variable in control subjects only and for all centres combined. Concentrations are expressed as nmol/l.

the RRs, such that only the C-peptide association remained marginally statistically significant (Table IV). Adjustment for waist circumference, and to a lesser extent, waist-hip ratio, had similar effects (data not shown).

When stratified by fasting status, levels of C-peptide, IGFBP-1 and IGFBP-2 were not associated with endometrial cancer risk among subjects whose blood was collected in the fasting state (>6 hr since last meal; crude p_{trend} = 0.97, 0.63 and 0.52, respectively), but were significantly associated with risk among subjects in the nonfasting state (≤6 hr; crude p_{trend} = 0.001, 0.04 and 0.001, respectively) (Fig. 2); however, formal tests for heterogeneity of the effect of fasting status on the association between peptide levels and endometrial cancer risk were not statistically significant ($p_{heterogeneity}$ for C-peptide = 0.36, IGFBP-1 = 0.23 and IGFBP-2 = 0.07, respectively). Among the nonfasting subjects, adjustment for BMI attenuated by about 20–40% the associations between peptide levels and risk; the association remained statistically significant for C-peptide and marginally significant for IGFBP-2, but not for IGFBP-1 (Fig. 2).

Similarly, the association between BMI and risk was moderately attenuated after adjustment for C-peptide, such that the RR for obese subjects (BMI of ≥ 30 kg/m^2) compared to normal weight subjects (BMI < 25 kg/m^2) was reduced from 2.37 [95% CI 1.60–3.51] to 1.93 [95% CI 1.27–2.92], but the association remained statistically significant (p_{trend} = 0.003). Although cases had a significantly lower mean level of physical activity than controls in our study (Table I), adjustment for physical activity level had negligible influence on the associations between peptide hormone levels and endometrial cancer risk, nor vice versa (data not shown).

To examine whether or not the association between hyperinsulinaemia and endometrial cancer risk is potentially mediated by SHBG and serum sex hormone levels, each hormone was included as a covariate in the crude regression model (data not shown). Adjustment for free estradiol had a strong attenuating effect on the association of C-peptide with endometrial cancer risk in postmenopausal women, such that the RR for the top *vs.* bottom quartile was reduced from 1.75 [95% CI 0.96–3.17] (p_{trend} = 0.04) to 1.28 [95% CI 0.67–2.45] (p_{trend} = 0.42). Adjustment for SHBG or free testosterone (in all women) had a weaker attenuating effect on the association between C-peptide and endometrial cancer risk (adjusted RR for the top *vs.* bottom quartile of C-peptide = 1.75 [95% CI 1.04–2.94], p_{trend} = 0.02, and 1.96 [95% CI 1.18–3.26], p_{trend} = 0.007, respectively). For the association between IGFBP-2 and endometrial cancer risk, SHBG had the strongest attenuating effect (adjusted RR for the top *vs.* bottom quartile of IGFBP-2 = 0.91 [95% CI 0.50–1.66], p_{trend} = 0.99), compared to adjustment for free estradiol in postmenopausal women (adjusted RR for the top *vs.* bottom quartile = 0.59 [95% CI 0.30–1.15], p_{trend} = 0.25) or free testosterone (adjusted RR for the top *vs.* bottom quartile = 0.77 [95% CI 0.44–1.34], p_{trend} = 0.47).

Exclusion of the 87 endometrial cancer cases diagnosed within the first 2 years of follow-up did not appreciably change the RR estimates for C-peptide or IGFBP-1 and cancer risk (data not shown). However, there was significant statistically heterogeneity in the association of IGFBP-2 and cancer risk between those diagnosed less than 2 years *vs.* 2 years or more since blood donation ($p_{heterogeneity}$ = 0.01), with a stronger inverse relationship between serum IGFBP-2 levels and risk among women diagnosed 2 years or more since blood donation (BMI-adjusted RR for the top *vs.* bottom quartile = 0.64 [95% CI 0.35–1.16], p_{trend} = 0.20).

All study results were similar when cut-points were specific for each centre rather than calculated for all centres combined, or when case-controls sets with known Type II histology (n = 9 cases) or with self-reported diabetes (n = 10 cases, 21 controls) were excluded (data not shown). There was some evidence of a stronger association between C-peptide levels and endometrial cancer risk among inactive women (below the median for MET-hr/wk of physical activity) compared to active women (above the median) ($p_{heterogeneity}$ = 0.02; RR for a doubling of C-peptide levels = 2.40 [95% CI 1.53–3.77], p = 0.0002, *vs.* 1.21 [95% CI 0.83–1.77], p = 0.31, respectively). There was no evidence that the associations between peptide hormones and endometrial cancer risk significantly differed by country, previous use of HRT, smoking, BMI, age at cancer diagnosis or menopausal status ($p_{heterogeneity}$ all > 0.05). The RR associated with a doubling of C-peptide, IGFBP-1 and IGFBP-2 levels among premenopausal women was 1.78 [95% CI 1.06–2.98], 1.07 [95% CI 0.81–1.41]

and 0.95 [95% CI 0.64–1.40], respectively, and among postmenopausal women was 1.62 [95% CI 1.20–2.18], 0.87 [95% CI 0.74–1.02] and 0.69 [95% CI 0.56–0.85], respectively ($p_{heterogeneity}$ = 0.76, 0.20 and 0.16, respectively).

Discussion

In this prospective study we found that women with elevated serum levels of C-peptide have an increased risk of subsequently developing endometrial cancer. This association remained marginally statistically significant after adjustment for BMI, suggesting that hyperinsulinaemia may be a risk factor for endometrial cancer independent of the effects of obesity. After accounting for BMI, IGFBP-1 levels were not associated with risk, and an inverse association between IGFBP-2 levels and risk was only evident among nonfasting women. Our results suggest that hyperinsulinaemia may influence endometrial cancer risk largely *via* its effects on endogenous sex hormones.

A major strength of our study is its prospective study design, in that the collection of blood samples and data on lifestyle factors was conducted prior to endometrial cancer diagnosis. This design minimizes biases that can affect studies with a traditional case-control design, due to differential recall of past exposures or to improper selection of control subjects. In addition, it reduces the possibility of 'reverse causation bias,' which may occur when the presence or diagnosis of a tumour modifies circulating peptide levels. Indeed, the stronger association of IGFBP-2 with endometrial cancer risk in cases diagnosed 2 years or more after blood collection suggests that the presence of a preclinical tumour, that can secrete IGFBP-2,[11,31] may have influenced circulating levels. Additional strengths included the extensive information on potential confounders and exclusion of women using exogenous hormones at blood collection, since they can alter circulating hormone levels.[32]

A limitation of our study was the lack of information on menopausal status at the time of cancer diagnosis. However, the RR estimates of the association between C-peptide and endometrial cancer did not substantially differ when stratified by menopausal status at baseline or by age at diagnosis (<55 and ≥55 years as a proxy for menopausal status at diagnosis), suggesting that insulin may be a risk factor in both pre- and post-menopausal women.

Laboratory variability was minimized by using 1 laboratory for analysis, and by measuring matched cases and controls within the same assay batch. However, a limitation of our study was the use of only 1 blood sample, taken at a single point in time, which does not perfectly reflect long-term circulating peptide hormone levels. Furthermore, there was a mixture of fasting and nonfasting samples in our study. Fasting levels are generally considered more reliable because nonfasting measurements of C-peptide and IGFBP-1 (but not IGFBP-2[11]) may be influenced by recent food intake, exercise and diurnal variation.[11,12,33] As such, we observed higher C-peptide levels, and lower IGFBP-1 levels, in nonfasting samples compared to fasting samples. Nevertheless, a number of other studies that have performed repeated measurements of blood serum samples over a 1–2-year period, among fasting and nonfasting, pre- and post-menopausal women, have reported correlations in the range of 0.58–0.62 for C-peptide levels, 0.63–0.89 for IGFBP-1 levels and 0.30–0.82 for IGFBP-2 levels, suggesting moderate to good reliability.[6,34,35] In addition, the study design incorporated matching of cases and controls according to fasting status (<3, 3–6, >6 hr) and time of day of blood collection (±1 hr) to minimize this variation.

Although insulin and C-peptide are secreted in equimolar amounts, C-peptide has a longer half-life than insulin and its circulating levels are subject to less fluctuation.[33] Thus, although C-peptide is still a short-term indicator of insulin production, C-peptide levels may be more representative of average circulating insulin levels, especially in nonfasting subjects.[33] However, a limitation of C-peptide is that it is primarily a marker of pancreatic insulin production rather than circulating insulin and so does not directly measure hyperinsulinaemia.

Although correlations of peptide levels were generally slightly stronger in fasting samples, C-peptide, IGFBP-1 and IGFBP-2 levels were associated with endometrial cancer risk in nonfasting subjects but not fasting subjects, and for IGFBP-1 only in the nonfasting crude analysis. This finding differs from previous studies that showed an association between fasting insulin levels and endometrial cancer risk,[7,8] or no difference in the associations for fasting (≥4 hr since last meal) and nonfasting (<4 hr) women.[6] Only a third of women in our study were 'fasting,' and the differential effect by fasting status may have occurred by chance because the tests for heterogeneity were not statistically significant. Alternatively, it is possible that postprandial serum C-peptide levels are a more sensitive measure of hyperinsulinaemia, since postprandial hyperinsulinaemia precedes the development of fasting hyperinsulinaemia,[36] and because hyperinsulinaemia is generally much more marked in the postprandial state than in the fasting state in people who are insulin resistant, obese, diabetic or elderly.[8,24,37,38] Prospective studies of colorectal[38] and breast cancer risk[21] have also shown that nonfasting serum insulin or C-peptide levels (rather than fasting levels) are associated with an increased risk. It is unknown whether high insulin level peaks during the postprandial phase are more aetiologically relevant to tumour development than raised 'fasting' insulin levels; however, if so, postprandial circulating insulin levels could have a disproportionate influence on endometrial cancer risk because humans spend most of their waking hours in the postprandial state.[23]

There are several mechanisms by which hyperinsulinaemia may be involved in the aetiology of endometrial cancer.[2] First, in the endometrium, insulin may directly stimulate cell proliferation and inhibit apoptosis by binding to insulin or IGF-I receptors.[39] Second, insulin can increase IGF-I bioactivity in the endometrium by directly inhibiting endometrial tissue gene expression of IGFBP-1, the most abundant IGF binding protein in the endometrium.[11,13,14,40] Third, insulin also reduces the hepatic synthesis and circulating levels of IGFBP-1 and IGFBP-2,[2,11,14] which is consistent with the negative correlation between circulating C-peptide levels and IGFBP-1 and IGFBP-2 levels seen in this and other studies.[41,42] Lower IGFBP-1 and IGFBP-2 levels may lead to increased IGF-I bioavailability in the circulation and endometrial tissue,[2,11,13,41,43] because contrary to IGFBP-3, IGFBP-1 and IGFBP-2 can combine with IGF-I to form a small complex that can cross the capillary endothelium and prevent IGF-I receptor binding.[11,12,14,40] Fourth, chronic hyperinsulinaemia is associated with polycystic ovary syndrome, a disorder in a genetically susceptible subgroup of premenopausal women that is characterized by ovarian androgen excess, chronic anovulation and progesterone deficiency,[2,44] and that is associated with an increased risk of endometrial cancer.[45]

Finally, and perhaps most importantly, insulin can increase the bioavailability of estradiol by down-regulating hepatic SHBG synthesis and up-regulating ovarian steroid hormone secretion.[2,4,46] This effect is consistent with observations by us and Troisi *et al.*,[7] that C-peptide was inversely correlated with SHBG and positively correlated with free estradiol. Most of the cases in our study were postmenopausal, among whom adjustment for free estradiol substantially attenuated the association between C-peptide and endometrial cancer risk. Indeed, elevated endogenous estrogen levels that are insufficiently counterbalanced by progesterone are thought to be the main aetiological factor leading to endometrial cancer (known as the unopposed estrogen hypothesis).[2,47] The findings from our study suggest that high insulin levels may increase endometrial cancer risk largely by reducing SHBG levels and increasing levels of unopposed free estradiol, although it is difficult to discern the specific effects of hyperinsulinaemia separate to other obesity-related effects on endogenous sex hormone levels.

The modest inverse association between IGFBP-1 levels and endometrial cancer risk among nonfasting women in our study,

although weak, is consistent with some,[6,15] but not all[8,16–18] previous epidemiological studies. Our finding that IGFBP-2 levels were more strongly inversely associated with endometrial cancer risk than IGFBP-1 levels is in contrast to the findings from the study by Lukanova *et al.*,[6] that found no association between IGFBP-2 levels and endometrial cancer risk and a strong inverse association for IGFBP-1 levels. Although all of these studies measured circulating levels of IGFBP-1 and/or IGFBP-2, the aetiological importance of serum *vs.* endometrial levels of IGFBP-1 and IGFBP-2 remains unclear. Little is known about the direct relationship between endometrial and serum levels of IGFBP-1 and IGFBP-2, however, serum (plasma) levels of IGFBP-1 and IGFBP-2 are definitely considered important regulators of the bioavailability of circulating IGF-I to various target tissues, including the endometrium.[11,12,14,40] Furthermore, although insulin is the primary determinant of IGFBP-1 levels,[14,43] in premenopausal women, IGFBP-1 gene expression in endometrial tissue is stimulated by progesterone in secretory-phase endometrium.[2,14,43] We did not measure progesterone levels in our study and thus could not examine a possible confounding effect of progesterone on IGFBP-1 levels among premenopausal women. Nevertheless, phase of menstrual cycle was a matching criterion in our study. Although the role of IGFBP-2 in cancer development is not clear, our results are consistent with the hypothesis that both IGFBP-1 and IGFBP-2 may serve to reduce the bioavailability of IGF-I in endometrial tissue. *In vitro* studies have also shown that IGFBP-2 inhibits IGF-II-stimulated mitogenesis and DNA synthesis by sequestering IGF-II and preventing it from binding to IGF receptors,[11,40] however, there is only weak epidemiologic evidence that IGF-II levels may be associated with endometrial cancer risk.[16,48]

Excess weight is an established risk factor for endometrial cancer and contributes directly to the development of insulin resistance.[2,4,49] The observation that adjustment for BMI only partly attenuated the association between C-peptide levels and cancer risk suggests that the link between hyperinsulinaemia and increased cancer risk is not totally explained by obesity. Further, the effect of visceral adiposity (waist circumference or waist-hip ratio) was similar to that of general obesity (BMI), which is consistent with evidence suggesting that visceral adiposity is no more important than subcutaneous fat in the development of insulin resistance.[50,51] Similarly, our finding that the association between obesity and endometrial cancer risk was only partly attenuated after adjustment for C-peptide is in agreement with the study by Lukanova *et al.*,[6] and suggests that the effect of obesity on endometrial cancer risk is only partly mediated by insulin.

Despite being a recognized risk factor for endometrial cancer and a marker for chronic hyperinsulinaemia,[9,10] the self-reported presence of Type II diabetes was not associated with risk in our study. However we had limited ability to examine this association because of the low prevalence (3.8%) of self-reported diabetes in our study, and the likelihood that diabetes was under-reported.[52] Although physical activity has been associated with improved insulin sensitivity, lower circulating insulin levels,[42,53] and increased levels of IGFBP-1[4,11,42] and SHBG,[3] physical activity was not correlated with any of these measures in our study. Further, the C-peptide, IGFBP-1 and IGFBP-2 risk estimates were not appreciably altered by adjustment for physical activity, and vice versa, indicating that the effects of physical activity on risk may not necessarily be mediated through the insulin pathway. However, our questionnaire assessment of physical activity may be insufficient to precisely assess energy expenditure. Our results suggest that physical activity may modify the association between hyperinsulinaemia and endometrial cancer risk because C-peptide levels were more strongly associated with risk in subjects who were relatively inactive.

In conclusion, our results provide modest support to the hypothesis that hyperinsulinaemia is a risk factor for endometrial cancer. Strategies to reduce the incidence of endometrial cancer should target changes in lifestyle factors that can improve insulin sensitivity and reduce serum insulin levels.[2–4,54,55]

Acknowledgements

We are very grateful to Ms. Nivonirina Robinot, Ms. Priscilia Amouyal and Ms. Josiane Bouzac for performing the laboratory analyses, Dr. Annekatrin Lukanova and Dr. Eva Lundin for coding the tumour morphology, Ms. Fatiha Louled for secretarial support, and all participants and staff involved in the EPIC study. Anne Cust received a PhD scholarship (UPA) from the University of Sydney and a Research Scholar Award from the Cancer Institute NSW, Australia.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide, IARC CancerBase No. 5. version 2.0. Lyon: IARC, 2004.
2. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev 2002;11:1531–43.
3. Vainio H, Bianchini F, eds.Weight control and physical activity—IARC handbook for cancer prevention, vol. 6. Lyon: IARC, 2002. 5–6.
4. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 2001;60:91–106.
5. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579–91.
6. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, Micheli A, Arslan AA, Rinaldi S, Muti P, Lenner P, Koenig KL, Biessy C, Krogh V, Riboli E, et al. Prediagnostic levels of C-peptide, IGF-I, IGFBP-1, -2 and -3 and risk of endometrial cancer. Int J Cancer 2004;108:262–8.
7. Troisi R, Potischman N, Hoover RN, Siiteri P, Brinton LA. Insulin and endometrial cancer. Am J Epidemiol 1997;146:476–82.
8. Rutanen EM, Stenman S, Blum W, Karkkainen T, Lehtovirta P, Stenman UH. Relationship between carbohydrate metabolism and serum insulin-like growth factor system in postmenopausal women: comparison of endometrial cancer patients with healthy controls. J Clin Endocrinol Metab 1993;77:199–204.
9. Anderson KE, Anderson E, Mink PJ, Hong CP, Kushi LH, Sellers TA, Lazovich D, Folsom AR. Diabetes and endometrial cancer in the Iowa women's health study. Cancer Epidemiol Biomarkers Prev 2001;10:611–6.
10. Weiderpass E, Persson I, Adami HO, Magnusson C, Lindgren A, Baron JA. Body size in different periods of life, diabetes mellitus, hypertension, and risk of postmenopausal endometrial cancer (Sweden). Cancer Causes Control 2000;11:185–92.
11. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 1997;18:801–31.
12. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocr Rev 1994;15:80–101.
13. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 2000;92:1472–89.
14. Lee PD, Giudice LC, Conover CA, Powell DR. Insulin-like growth factor binding protein-1: recent findings and new directions. Proc Soc Exp Biol Med 1997;216:319–57.
15. Ayabe T, Tsutsumi O, Sakai H, Yoshikawa H, Yano T, Kurimoto F, Taketani Y. Increased circulating levels of insulin-like growth factor-I and decreased circulating levels of insulin-like growth factor binding protein-1 in postmenopausal women with endometrial cancer. Endocr J 1997;44:419–24.
16. Petridou E, Koukoulomatis P, Alexe DM, Voulgaris Z, Spanos E, Trichopoulos D. Endometrial cancer and the IGF system: a case-control study in Greece. Oncology 2003;64:341–5.
17. Weiderpass E, Brismar K, Bellocco R, Vainio H, Kaaks R. Serum levels of insulin-like growth factor-I, IGF-binding protein 1 and 3, and insulin and endometrial cancer risk. Br J Cancer 2003;89:1697–704.
18. Augustin LS, Dal Maso L, Franceschi S, Talamini R, Kendall CW, Jenkins DJ, Vidgen E, La Vecchia C. Association between components of the insulin-like growth factor system and endometrial cancer risk. Oncology 2004;67:54–9.
19. Lukanova A, Soderberg S, Stattin P, Palmqvist R, Lundin E, Biessy C, Rinaldi S, Riboli E, Hallmans G, Kaaks R. Nonlinear relationship of insulin-like growth factor (IGF)-I and IGF-I/IGF-binding protein-3 ratio with indices of adiposity and plasma insulin concentrations (Sweden). Cancer Causes Control 2002;13:509–16.

20. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
21. Verheus M, Peeters PH, Rinaldi S, Dossus L, Biessy C, Olsen A, Tjonneland A, Overvad K, Jeppesen M, Clavel-Chapelon F, Tehard B, Nagel G, et al. Serum C-peptide levels and breast cancer risk: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 2006;119:659–67.
22. Haftenberger M, Schuit AJ, Tormo MJ, Boeing H, Wareham N, Bueno-de-Mesquita HB, Kumle M, Hjartaker A, Chirlaque MD, Ardanaz E, Andren C, Lindahl B, et al. Physical activity of subjects aged 50–64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5:1163–76.
23. Ahmed M, Gannon MC, Nuttall FQ. Postprandial plasma glucose, insulin, glucagon and triglyceride responses to a standard diet in normal subjects. Diabetologia 1976;12:61–7.
24. Skilton MR, Lai NT, Griffiths KA, Molyneaux LM, Yue DK, Sullivan DR, Celermajer DS. Meal-related increases in vascular reactivity are impaired in older and diabetic adults: insights into roles of aging and insulin in vascular flow. Am J Physiol Heart Circ Physiol 2005;288: H1404–H1410.
25. Tavassoli FA, Devilee P, eds. Pathology and genetics—tumours of the breast and female genital organs. World health organization classification of tumours. Lyon: IARC, 2003. 217–258.
26. Emons G, Fleckenstein G, Hinney B, Huschmand A, Heyl W. Hormonal interactions in endometrial cancer. Endocr Relat Cancer 2000;7: 227–42.
27. Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, Secreto G, Amiano P, Bingham S, Boeing H, Bueno de Mesquita HB, Chang-Claude J, et al. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2005;97:755–65.
28. Rinaldi S, Dechaud H, Biessy C, Morin-Raverot V, Toniolo P, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Secreto G, Ciampi A, Riboli E, Kaaks R. Reliability and validity of commercially available, direct radioimmunoassays for measurement of blood androgens and estrogens in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:757–65.
29. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Riboli E, Toniolo P, Kaaks R. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev 2002;11:1065–71.
30. Richardson DB. An incidence density sampling program for nested case-control analyses. Occup Environ Med 2004;61:e59.
31. Zumkeller W, Schwander J, Mitchell CD, Morrell DJ, Schofield PN, Preece MA. Insulin-like growth factor (IGF)-I, -II and IGF binding protein-2 (IGFBP-2) in the plasma of children with Wilms' tumour. Eur J Cancer A 1993;29:1973–7.
32. Beral V, Bull D, Reeves G. Endometrial cancer and hormone-replacement therapy in the Million Women Study. Lancet 2005;365:1543–51.
33. Bonser AM, Garcia-Webb P. C-peptide measurement: methods and clinical utility. Crit Rev Clin Lab Sci 1984;19:297–352.
34. Kaaks R, Toniolo P, Akhmedkhanov A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, Zeleniuch-Jacquotte A, Shore RE, Riboli E. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst 2000; 92:1592–600.
35. Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, Ram M, Freudenheim JL, Sieri S, Trevisan M, Berrino F. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2002;11:1361–8.
36. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415–28.
37. Emberson JR, Whincup PH, Walker M, Thomas M, Alberti KG. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002;39:493–501.
38. Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, Tracy RP, Dobs A, Savage PJ. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999;91:1147–54.
39. Nagamani M, Stuart CA. Specific binding and growth-promoting activity of insulin in endometrial cancer cells in culture. Am J Obstet Gynecol 1998;179:6–12.
40. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
41. Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, Lee HC, Huh KB. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. Int J Obes Relat Metab Disord 1997;21:355–9.
42. Allen NE, Appleby PN, Kaaks R, Rinaldi S, Davey GK, Key TJ. Lifestyle determinants of serum insulin-like growth-factor-I (IGF-I), C-peptide and hormone binding protein levels in British women. Cancer Causes Control 2003;14:65–74.
43. Rutanen EM, Nyman T, Lehtovirta P, Ammala M, Pekonen F. Suppressed expression of insulin-like growth factor binding protein-1 mRNA in the endometrium: a molecular mechanism associating endometrial cancer with its risk factors. Int J Cancer 1994;59:307–12.
44. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18: 774–800.
45. Wild S, Pierpoint T, Jacobs H, McKeigue P. Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. Hum Fertil (Camb) 2000;3:101–5.
46. Yki-Jarvinen H, Makimattila S, Utriainen T, Rutanen EM. Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 in vivo. J Clin Endocrinol Metab 1995;80:3227–32.
47. Key TJ, Pike MC. The dose-effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. Br J Cancer 1988;57:205–12.
48. Oh JC, Wu W, Tortolero-Luna G, Broaddus R, Gershenson DM, Burke TW, Schmandt R, Lu KH. Increased plasma levels of insulin-like growth factor 2 and insulin-like growth factor binding protein 3 are associated with endometrial cancer risk. Cancer Epidemiol Biomarkers Prev 2004;13:748–52.
49. Bergman RN, Mittelman SD. Central role of the adipocyte in insulin resistance. J Basic Clin Physiol Pharmacol 1998;9:205–21.
50. Miles JM, Jensen MD. Counterpoint: visceral adiposity is not causally related to insulin resistance. Diabetes Care 2005;28:2326–8.
51. Frayn KN. Visceral fat and insulin resistance-causative or correlative? Br J Nutr 2000;83 (Suppl. 1):S71–S77.
52. The Decode Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. Diabetes Care 2003;26:61–9.
53. Borghouts LB, Keizer HA. Exercise and insulin sensitivity: a review. Int J Sports Med 2000;21:1–12.
54. Chlebowski RT, Pettinger M, Stefanick ML, Howard BV, Mossavar-Rahmani Y, McTiernan A. Insulin, physical activity, and caloric intake in postmenopausal women: breast cancer implications. J Clin Oncol 2004;22:4507–13.
55. Torjesen PA, Birkeland KI, Anderssen SA, Hjermann I, Holme I, Urdal P. Lifestyle changes may reverse development of the insulin resistance syndrome. The Oslo Diet and Exercise Study: a randomized trial. Diabetes Care 1997;20:26–31.