Original Contribution

A Prospective Study of Insulin-Like Growth Factor 1, Its Binding Protein 3, and Risk of Endometriosis

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Several retrospective case-control studies suggested that insulin-like growth factor 1 (IGF-1) or insulin-like growth factor binding protein 3 (IGFBP-3) was associated with endometriosis. However, results are inconsistent and no prospective study exists. We prospectively evaluated associations between plasma levels of IGF-1 and IGFBP-3 and laparoscopically confirmed endometriosis in a case-control study nested within the Nurses' Health Study II. Between blood collections in 1996–1999 and 2007, we ascertained 310 premenopausal women with incident endometriosis and 615 matched controls. We estimated incidence rate ratios and 95% confidence intervals using multivariable conditional logistic regression. We observed no statistically significant associations between endometriosis and IGF-1 (incidence rate ratio (IRR) = 0.88, 95% confidence interval (CI): 0.61, 1.27; $P_{trend} = 0.48$), IGFBP-3 (IRR = 1.12, 95% CI: 0.80, 1.57; $P_{trend} = 0.51$), and the IGF-1:IGFBP-3 molar ratio (IRR = 0.94, 95% CI: 0.66, 1.34; $P_{trend} = 0.64$), comparing the top with the bottom tertile. IGF-1, IGFBP-3, and the molar ratio appeared to be positively associated with endometriosis risk among women aged <40 years at blood draw (IGF-1: IRR = 1.60, 95% CI: 0.86, 2.98; IGFBP-3: IRR = 1.85, 95% CI: 1.08, 3.16; IGF-1:IGFBP-3: IRR = 1.57, 95% CI: 0.85, 2.88) but not among women aged ≥40 years at blood draw (all $P_{heterogeneity} \leq 0.05$). Overall, these data suggest that, if IGF-1 or IGFBP-3 plays a role in the etiology of endometriosis, it is minimal and perhaps only among younger women.

endometriosis; insulin-like growth factor 1; insulin-like growth factor binding protein 3

Abbreviations: BMI, body mass index; CI, confidence interval; IGF, insulin-like growth factor; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IRR, incidence rate ratio; NHSII, Nurses' Health Study II.

Endometriosis is a benign, chronic, and estrogen-dependent gynecological disorder that affects 6%–10% of women of reproductive age in the United States (1). It is defined as the presence of endometrium-like tissue in the sites outside the uterine cavity, primarily on the pelvic peritoneum and ovaries (2). Common signs and symptoms include chronic pelvic pain, dysmenorrhea, dyspareunia, and reduced fertility (1). Sampson's theory (3) suggests that endometriotic implants originate from retrograde menstruation; that is, during menstrual shedding, endometrial cells from the endometrium reach their most common site of implantation, the peritoneal cavity, by traveling through the fallopian tubes. Retrograde menstruation is present in most women, but only a minority of women develop endometriosis; hence, other fundamental processes must contribute to the implantation of endometrial cells outside the uterine cavity and their subsequent proliferation and progression into endometriotic lesions (1, 4).

Insulin-like growth factor 1 (IGF-1) is a peptide hormone, which is bound to a family of insulin-like growth factor binding proteins that regulate its effects and bind to specific receptors on target cells (5). IGF-1 promotes cellular growth and differentiation, and it is one of the several possible mediators of estrogen and other growth factors' signaling pathways in various body tissues (6, 7). The various binding proteins inhibit, as well as augment, insulin-like growth factor (IGF) action in a variety of systems (6).

The IGF system has been implicated in the pathogenesis of endometriosis. The presence of the IGF system was observed in human peritoneal fluid (8, 9), and expression and localization of IGF-1 and its binding proteins were demonstrated in both the eutopic endometrium and in endometriotic lesions

Table 1. Age-Standardized Characteristics of Endometriosis Cases and Matched Controls[a] in the Nurses' Health Study II, 1996–2007

Characteristic	Cases ($n = 310$)		Controls ($n = 615$)	
	Mean (SD)	%	Mean (SD)	%
Age at blood draw, years	40.9 (4.1)		41.4 (4.1)	
White race		97		96
Age at menarche, years[b]				
<12		25		20
12		35		31
>12		40		49
BMI[c] at age 18 years				
<18.5		15		15
18.5–22.4		63		60
22.5–25		13		16
>25		10		9
BMI[c] at blood draw				
<22.5		35		36
22.5–24.9		21		23
25–29.9		24		23
≥30.0		21		17
Height, m	1.7 (0.1)		1.7 (0.1)	
No. of pregnancies >6 months				
Nulliparous		30		21
1–2		49		50
≥3		22		29
Months of breastfeeding[d]				
0 or <1		16		13
<12		31		34
12–24		30		27
>24		22		26
Infertility history		27		30
Oral contraceptive use				
Never		18		15
Past		82		85
Smoking history				
Never		71		65
Past		22		22
Current		7		13
Alternative Health Eating Index 2010, score[e]	49.5 (10.7)		49.5 (10.6)	
Alcohol intake, g/day	1.8 (0.7)		1.8 (0.8)	
Physical activity, METs/week[f]	2.8 (1.1)		2.8 (1.1)	
IGF-1, ng/mL	263 (87)		266 (85)	
IGFBP-3, ng/mL	5,303 (996)		5,284 (993)	
IGF-1:IGFBP-3 molar ratio[g]	0.2 (0.0)		0.2 (0.0)	

Abbreviations: BMI, body mass index; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; MET, metabolic equivalent; SD, standard deviation.

[a] Laparoscopically confirmed cases of endometriosis were matched 1:2 with controls of comparable age, race, infertility history, and menopausal status at diagnosis, as well as month, time of day, and fasting status at blood draw.

[b] Values of polytomous variables may not sum to 100% because of rounding.

[c] Weight (kg)/height $(m)^2$.

[d] Among parous women.

[e] The Alternative Health Eating Index 2010 (AHEI-2010) is a score that measures adherence to a diet pattern based on foods and nutrients most predictive of disease risk in the literature; range 0–110.

[f] Metabolic equivalents from recreational and leisure-time activities.

[g] The IGF-1:IGFBP-3 molar ratio is the quantity (IGF-1, ng/mL, ×0.130)/(IGFBP-3, ng/mL, ×0.036) where the constants are the molecular weights of the respective polypeptide.

(10). In addition, it has been demonstrated that IGFs are mitogenic to endometrial stromal cell cultures in vitro (8). Early case-control studies showed that there was a positive association between IGF-1 levels and endometriosis (5, 11). However, more recent studies found no association (12, 13). Studies on the association between insulin-like growth factor binding protein 3 (IGFBP-3) and endometriosis were also inconsistent (12–14). Moreover, no prospective study has been conducted, which is essential to provide temporal evidence for these associations. Therefore, we conducted a matched case-control study, nested within the ongoing prospective Nurses' Health Study II (NHSII) cohort, to prospectively investigate associations of IGF-1 or IGFBP-3 with endometriosis risk.

METHODS

Study population and data collection

Data for this study were collected in the NHSII cohort from September 1989 to June 2007. A total of 116,430 female registered nurses, ranging in age from 25 to 42 years and residing in 1 of 14 states in the United States, completed a baseline questionnaire in 1989. Questionnaires requesting information on incidence of diseases and demographical, biological, environmental, and lifestyle risk factors are updated and mailed biennially. Subsequent follow-up of this cohort in each 2-year interval has been consistently ≥90%. This research was approved by the Institutional Review Board of Brigham and Women's Hospital and Harvard School of Public Health, Boston, Massachusetts.

Establishment of the nested blood cohort has been described in detail previously (15). In brief, blood samples were collected between 1996 and 1999 from 29,611 members of this cohort who were between the ages of 32 and 53 years at the time of blood draw. Premenopausal women who had not taken hormones and who had not been pregnant or breastfed within 6 months ($n = 18,521$) provided timed blood samples on days 3–5 of their menstrual cycle (follicular) and 7–9 days before the anticipated start of their next cycle (luteal). All other women ($n = 11,090$) provided a single untimed (random)

Table 2. Incidence Rate Ratios and 95% Confidence Intervals of Laparoscopically Confirmed Endometriosis by Tertiles of IGF-1 and IGFBP-3 in the Nurses' Health Study II, 1996–2007

Protein and Model	Tertile 1				Tertile 2				Tertile 3				P_{trend}[a]
	No. of Cases	No. of Controls	IRR	95% CI	No. of Cases	No. of Controls	IRR	95% CI	No. of Cases	No. of Controls	IRR	95% CI	
IGF-1[b]	104	205			113	205			93	205			
Base model A[c]			1.00	Referent			1.08	0.78, 1.50			0.88	0.61, 1.27	0.48
Base model B[d]			1.00	Referent			1.03	0.73, 1.46			0.78	0.51, 1.18	0.24
Multivariable[e]			1.00	Referent			1.09	0.76, 1.58			0.93	0.62, 1.40	0.70
IGFBP-3[f]	100	205			99	205			111	205			
Base model A			1.00	Referent			0.99	0.70, 1.40			1.12	0.80, 1.57	0.51
Base model B			1.00	Referent			1.04	0.73, 1.49			1.27	0.86, 1.87	0.23
Multivariable			1.00	Referent			1.03	0.72, 1.48			1.14	0.80, 1.64	0.47
IGF-1:IGFBP-3[g,h]	116	205			86	205			108	205			
Base model[b]			1.00	Referent			0.75	0.53, 1.05			0.94	0.66, 1.34	0.64
Multivariable			1.00	Referent			0.76	0.52, 1.10			1.05	0.71, 1.55	0.87

Abbreviations: CI, confidence interval; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IRR, incidence rate ratio.

[a] P_{trend} calculated by the Wald test of a score variable that contained median values of tertiles.

[b] IGF-1 tertile 1 is <222 ng/mL; tertile 2 is 222–300 ng/mL; tertile 3 is ≥301 ng/mL.

[c] Incidence rate ratios and 95% confidence intervals were estimated by conditional logistic regression conditioned on the matching factors age, race, infertility history, and menopausal status at diagnosis, as well as month, time of day, and fasting status at blood draw.

[d] Base model A further adjusted for plasma IGF-1 or IGFBP-3.

[e] Base model B further adjusted for age at menarche, body mass index at age 18 years and at blood draw, height, number of pregnancies >6 months, total months of breastfeeding, and physical activity.

[f] IGFBP-3 tertile 1 is <4,807 ng/mL; tertile 2 is 4,807–5,644 ng/mL; tertile 3 is ≥5,645 ng/mL.

[g] IGF-1:IGFBP-3 tertile 1 is <0.16; tertile 2 is 0.16–0.19; tertile 3 is ≥0.20.

[h] IGF-1:IGFBP-3, molar ratio of IGF-1 to IGFBP-3, is the quantity (IGF-1, ng/mL, × 0.130)/(IGFBP-3, ng/mL, × 0.036) where the constants are the molecular weights of the respective polypeptide.

blood sample. Samples were shipped with an ice pack via overnight courier to the laboratory where they were processed and separated into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored, liquid nitrogen freezers since collection.

Each woman completed a brief questionnaire at blood draw that recorded the date and time of day the blood sample was drawn, her current weight, current parity, current smoking status, current alcohol use, medication use, hours since last food intake, any recent changes in the character of her menstrual cycle, and the first day of the menstrual cycle in which the blood samples were drawn. Each woman was also provided a postcard to indicate the first day of her menstrual cycle following the blood collection. Follow-up of the blood cohort was over 96% in 2005. We restricted the analysis to premenopausal women at blood draw, who were defined as 1) reporting that their periods had not ceased or 2) had a hysterectomy but had at least 1 ovary remaining and were aged ≤47 (for nonsmokers) or ≤45 (for smokers) years of age. We used luteal and random samples in this study, because cyclic variations of IGF are only modest (16, 17).

Case ascertainment

Cases of endometriosis were restricted to those women who reported laparoscopic confirmation of the diagnosis and were diagnosed at least 1 year after blood draw. Women who had hysterectomy were censored at the questionnaire cycle of hysterectomy. As previously reported (18), the surgical report case validation for self-reported, laparoscopically confirmed endometriosis cases within this cohort is 96%. Women were first asked if they "ever had physician-diagnosed endometriosis" and, if so, whether the diagnosis was confirmed by laparoscopy and when the diagnosis occurred. The same questions were asked in each subsequent questionnaire cycle. Blood samples were collected from 29,611 women between 1996 and 1999 as described above. Of these, 363 women were diagnosed with laparoscopically confirmed endometriosis at least 1 year after blood draw. The final analytical data set included 310 cases of endometriosis. Reasons for exclusion (n =53) included being postmenopausal at blood draw, inadequate sample volume, or inability to adequately match with a control specimen.

Control selection

Women with a prior cancer (except nonmelanoma skin cancer) or hysterectomy in any cycle before or during the cycle in which a case was diagnosed or who did not have enough blood volume (≤1.8 mL) were not eligible to serve as controls. For each incident case of laparoscopically confirmed endometriosis, 2 controls were randomly selected from the risk set who were of the same race (white, Asian, African American, Hispanic, other) and age (±1 year) and had the same infertility

history (ever/never) and menopausal status at diagnosis (premenopausal/postmenopausal/unknown). Cases and controls were also matched on the month (±1 month), time of day (±2 hours), and fasting status (<2, 2–4, 5–7, 8–11, >12 hours' fasting) at blood draw. By design, if a woman who was sampled as a control later became a case and satisfied the case inclusion criteria, she was also included as a case. Assessment of exposure (IGF assays) is discussed in Appendix 1.

Statistical analyses

Tertiles of IGF-1 and IGFBP-3 levels were defined on the basis of plasma levels of all controls. IGF-1 was assessed in the models with and without IGFBP-3 as a factor, because there is evidence that IGFBP-3 may decrease IGF-1 bioactivity via direct or indirect mechanisms (19). Conditional logistic regression models were used to estimate the incidence rate ratios (20) and 95% confidence intervals. In addition to the matching factors, we also adjusted for other potential confounders including age at menarche (<12, 12, >12 years), body mass index (BMI, expressed as weight (kg)/height $(m)^2$) at age 18 years (<18.5, 18.5–22.4, 22.5–25.0, >25), BMI at blood draw (<22.5, 22.5–24.9, 25.0–29.9, ≥30), height (<1.60, 1.60–1.64, 1.65–1.69, ≥1.70 m), number of pregnancies of >6 months (0, 1–2, >2 births), total months of breast feeding (nulliparous, 0 or <1, 1–11, 12–23, ≥24 months), and physical activity (metabolic equivalents) from recreational and leisure-time activities (<3, 3 to <9, 9 to <18, ≥18 metabolic equivalent-hours/week). Other factors were considered, but because they altered incidence rate ratios for IGF and IGFBP-3 by less than 2%, they were not included in the final models (past oral contraceptive use, diet (Alternative Health Eating Index–2010) (21), smoking history, and alcohol consumption). We tested for trends by calculating the Wald statistic for medians of tertiles. We examined whether the association between IGFs and endometriosis varied by age at blood draw (<40 or ≥40 years), age at diagnosis of endometriosis (<45 or ≥45 years), BMI at age 18 years (<20 or ≥20), BMI at blood draw (<25 or ≥25), and infertility history at diagnosis (ever or never), using conditional logistic regression. We assessed the statistical significance of the interactions between IGFs and those subgroup variables via the likelihood ratio test comparing the model with main effects only with the model with main effects and interaction terms. Unconditional logistic regression adjusting for matching variables were also conducted for both main analysis and subgroup analysis. To evaluate the impact of adjusting for adulthood BMI in the multivariable model, we conducted sensitivity analyses that did not adjust for adulthood BMI. To account for diagnostic delay, we conducted subanalyses excluding endometriosis cases diagnosed within 2 or 4 years after blood draw.

All *P* values reported are for a 2-sided test. Analyses were performed by using Statistical Analysis Software (SAS), version 9.3 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

A total of 310 women with incident laparoscopically confirmed endometriosis and 615 controls comprised the study population. Age-standardized characteristics were compared between cases and matched controls (Table 1). The mean age at blood draw was 41 years for both cases and controls, with a range of 33–51 years among cases and 32–51 years among controls. The median time between blood collection and diagnosis was 4 years with an interquartile range of 3–6 years. Because cases and controls were matched by age, race, and infertility history, the distributions of these variables are comparable by design (Table 1). The majority of the study population was Caucasian. Overall, cases had an earlier age at menarche, were more likely to be nulliparous, had shorter durations of breastfeeding, and were less likely to smoke compared with controls. Mean levels of IGF-1, IGFBP-3, and molar ratio IGF-1:IGFBP-3 were very similar between cases and controls.

Overall, circulating IGF-1 levels were not associated with endometriosis risk (incidence rate ratio (IRR) = 0.88, 95% confidence interval (CI): 0.61, 1.27; $P_{trend} = 0.48$), when comparing the top with the bottom tertile. When additionally adjusting for IGFBP-3 levels or further adjusting for age at menarche, BMI at age 18 years and at blood draw, height, parity, duration of lactation, and physical activity, the results were similar (Table 2). Circulating IGFBP-3 and the molar ratio IGF-1:IGFBP-3 were not associated with endometriosis risk (IGFBP-3: IRR = 1.12, 95% CI: 0.80, 1.57; $P_{trend} = 0.51$; molar ratio IGF-1:IGFBP-3: IRR = 0.94; 95% CI: 0.66, 1.34; $P_{trend} = 0.64$) when comparing the top with the bottom tertile. Further adjustment for IGF-1 (for IGFBP-3) or additional covariates did not change the results (Table 2). When unconditional logistic regressions adjusted for all matching factors were used, the results remained largely unchanged (data not shown).

We stratified by age at blood draw, age at diagnosis of endometriosis, BMI at age 18 years, BMI at blood draw, and infertility history at diagnosis to evaluate whether the associations between IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio and endometriosis differed among subgroups. We found heterogeneity by age at blood draw for IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar ratio (all $P_{heterogeneity} \leq 0.05$). IGF-1, IGFBP-3, and the molar ratio IGF-1:IGFBP-3 seemed to be associated with endometriosis risk among women aged <40 years at blood draw (top vs. bottom tertile, IGF-1: IRR = 1.6, 95% CI: 0.86, 2.98; IGFBP-3: IRR = 1.85, 95% CI: 1.08, 3.16; IGF-1:IGFBP-3 molar ratio: IRR = 1.57, 95% CI: 0.85, 2.88) but not among women aged ≥40 years at blood draw (Table 3). The lack of associations between IGFs and endometriosis risk remained unchanged when evaluated within strata of age at diagnosis of endometriosis, BMI at age 18 years, BMI at blood draw, and infertility history at diagnosis. Although we found statistically significant interactions among BMI at blood draw, IGF-1, and the IGF-1:IGFBP-3 molar ratio, no consistent patterns among the stratum-specific incidence rate ratios emerged (Table 3). When further adjusting for age at menarche, BMI at age 18 years and at blood draw, height, parity, duration of lactation, and physical activity, we found that the results remained largely unchanged (data not shown). When unconditional logistic regressions adjusted for all matching factors were used, the results remained similar except that the positive associations between IGFs and endometriosis among women younger than age 40 years at blood draw were attenuated (data not

Table 3. Incidence Rate and 95% Confidence Intervals[a] for Endometriosis by Tertiles of IGF-1 and IGFBP-3 Among Subgroups in the Nurses' Health Study II, 1996–2007

Protein and Subgroup	Tertile 2		Tertile 3		P_{trend}[b]	$P_{interaction}$[c]
	IRR	95% CI	IRR	95% CI		
Age at Blood Draw, years[d]						
IGF-1						<0.001
<40	2.70	1.49, 4.90	1.60	0.86, 2.98	0.25	
≥40	0.75	0.51, 1.11	0.75	0.48, 1.16	0.09	
IGFBP-3						0.05
<40	1.49	0.83, 2.70	1.85	1.08, 3.16	0.07	
≥40	0.85	0.57, 1.27	0.88	0.58, 1.32	0.85	
IGF-1:IGFBP-3[e]						0.03
<40	1.45	0.77, 2.73	1.57	0.85, 2.88	0.24	
≥40	0.59	0.39, 0.88	0.80	0.52, 1.24	0.24	
Age at Diagnosis, years[f]						
IGF-1						0.25
<45	1.60	0.88, 2.89	1.29	0.71, 2.37	0.52	
≥45	0.91	0.60, 1.38	0.76	0.47, 1.24	0.27	
IGFBP-3						0.65
<45	1.09	0.60, 1.97	1.42	0.80, 2.52	0.21	
≥45	1.00	0.64, 1.55	1.03	0.66, 1.59	0.91	
IGF-1:IGFBP-3						0.15
<45	1.24	0.67, 2.31	1.28	0.72, 2.28	0.47	
≥45	0.59	0.39, 0.91	0.89	0.55, 1.43	0.35	
BMI at Age 18 Years[g,h]						
IGF-1						0.12
<20	0.89	0.51, 1.55	1.07	0.61, 1.89	0.69	
≥20	1.29	0.84, 1.99	0.75	0.48, 1.19	0.24	
IGFBP-3						0.53
<20	0.94	0.56, 1.57	1.31	0.77, 2.23	0.30	
≥20	1.03	0.67, 1.61	1.00	0.65, 1.54	0.99	
IGF-1:IGFBP-3						0.20
<20	0.69	0.40, 1.21	1.20	0.69, 2.09	0.43	
≥20	0.84	0.54, 1.31	0.78	0.50, 1.22	0.24	

Table continues

shown). Multivariable results remained largely unchanged when adjustment for adulthood BMI was not included (data not shown).

To account for the possibility that endometriosis had been present prior to diagnosis by laparoscopy, we conducted subanalyses excluding endometriosis cases diagnosed within 2 years or 4 years after blood draw. There remained no association between IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar ratio and endometriosis risk in multivariable models ($P_{trend} \geq 0.36$ for all comparisons, data not shown). The stratified results remained essentially unchanged in multivariable models when excluding endometriosis cases diagnosed within 2 years after blood draw, but the stratified results by age at blood draw were attenuated when excluding cases diagnosed within 4 years after blood draw (data not shown).

DISCUSSION

Overall, we found no association between IGF-1, IGFBP-3 level, and the molar ratio IGF-1:IGFBP-3 and endometriosis risk in this prospective, matched, nested case-control study. However, our data suggest that the associations may vary by age at blood draw, with an increased endometriosis risk among women who were younger than 40 years at blood draw.

Previous case-control studies that evaluated associations between serum or peritoneal fluid IGF-1 or IGFBP-3 levels and endometriosis risk reported inconsistent findings (5, 11–14, 22). Gurgan et al. (5) investigated the association between serum and peritoneal fluid IGF-1 and IGFBP-3 levels among 44 women and found that mean serum and peritoneal IGF-1 levels of controls and early-stage endometriosis cases

Table 3. Continued

Protein and Subgroup	Tertile 2		Tertile 3		P_{trend}[b]	$P_{interaction}$[c]
	IRR	95% CI	IRR	95% CI		
BMI at Blood Draw[h,i]						
IGF-1						0.001
<25	0.95	0.59, 1.52	1.19	0.73, 1.93	0.38	
≥25	1.59	0.96, 2.62	0.49	0.27, 0.88	0.06	
IGFBP-3						0.21
<25	0.94	0.60, 1.46	1.32	0.84, 2.07	0.19	
≥25	1.10	0.65, 1.89	0.87	0.52, 1.45	0.57	
IGF-1:IGFBP-3						0.01
<25	0.70	0.43, 1.15	1.23	0.76, 1.98	0.24	
≥25	0.96	0.59, 1.59	0.58	0.33, 1.03	0.07	
Infertility History[j,k]						
IGF-1						0.40
Never	1.19	0.79, 1.79	1.04	0.67, 1.61	0.99	
Ever	0.89	0.50, 1.59	0.60	0.31, 1.17	0.19	
IGFBP-3						0.22
Never	1.24	0.81, 1.91	1.22	0.81, 1.85	0.47	
Ever	0.67	0.38, 1.18	0.99	0.55, 1.78	0.86	
IGF-1:IGFBP-3						0.66
Never	0.81	0.54, 1.21	1.04	0.67, 1.59	0.91	
Ever	0.62	0.33, 1.17	0.75	0.39, 1.42	0.47	

Abbreviations: BMI, body mass index; CI, confidence interval; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IRR, incidence rate ratio.

[a] All incidence rate ratios and 95% confidence intervals were estimated by conditional logistic regression conditioned on the matching factors including age, race, infertility history, and menopausal status at diagnosis, as well as month, time of day, and fasting status at blood draw.

[b] P_{trend} calculated by the Wald test of a score variable that contained median values of tertiles.

[c] $P_{interaction}$ calculated from likelihood ratio tests of the interactions terms.

[d] There were 115 cases and 204 controls in the age at blood draw <40 years category and 195 cases and 411 controls in the ≥40 years category.

[e] IGF-1:IGFBP-3, molar ratio of IGF-1 to IGFBP-3, is the quantity (IGF-1, ng/mL, × 0.130)/(IGFBP-3, ng/mL, × 0.036) where the constants are the molecular weights of the respective polypeptide.

[f] There were 127 cases and 213 controls in the age at diagnosis <45 years category and 183 cases and 402 controls in the ≥45 years category.

[g] There were 124 cases and 253 controls in the BMI at age 18 <20 years category and 186 cases and 362 controls in the ≥20 years category.

[h] Weight (kg)/height $(m)^2$.

[i] There were 177 cases and 368 controls in the BMI at blood draw <25 category and 133 cases and 247 controls in the ≥25 category.

[j] There were 209 cases and 414 controls in the infertility history never category and 101 cases and 201 controls in the infertility history ever category.

[k] Infertility history at diagnosis of laparoscopically confirmed endometriosis.

were significantly lower than those of late-stage cases, while there were no significant differences in mean IGFBP-3 levels between the groups. Kim et al. (11) studied peritoneal IGF-1 and IGFBP-3 among 43 women and found that mean IGF-1 levels were significantly higher in cases than in controls, whereas mean IGFBP-3 levels were significantly lower in cases than in controls. However, more recent case-control studies with slightly larger sample sizes have found no association of serum IGF-1 or IGFBP-3 with endometriosis (total $n = 48$, 72, and 148, respectively) (12–14).

These inconsistent findings could be accounted for by low statistical power, inappropriate control selection, and absence of adjustment for confounders. The sample size of those studies was generally quite small, which made the results susceptible to random error. Moreover, these case-control studies selected controls from either women without pathology undergoing tubal ligation (proven to be fertile) or from women who had pathology that prompted the need for laparoscopy but had no evidence of endometriosis on laparoscopy. Such study design issues may bias associations in an unpredictable

direction, because the results may have been driven by underlying causes for infertility or other pathology that prompted the need for laparoscopy rather than driven by endometriosis.

The present study suggests that circulating levels of IGF-1 and IGFBP-3 and the IGF-1:IGFBP-3 molar ratio were associated with higher risk of endometriosis among women younger than 40 years at blood draw but not among women older than 40 years at blood draw. The elevated risks seen among younger women were attenuated when endometriosis cases within 4 years after blood draw were excluded. This indicates that the association could have been driven by undiagnosed endometriosis at blood draw, where the elevated IGF levels were results of the disease process rather than the initiators of the disease. It is possible that the IGF-specific changes caused by disease progression were distinct for younger and older women. However, the attenuation with the 4 years' exclusion may be due to the much lower power to detect the potential positive association (50% of endometriosis cases were excluded). Thus, this heterogeneity can also be explained by the potential different pathogenesis and risk profile between endometriosis that is clinically evident among younger women and endometriosis that first becomes clinically evident at an older age.

A key strength of this study is that blood samples were collected, on average, 4 years prior to endometriosis diagnosis. The prospective study design uniquely allows evaluation of the temporality between IGF levels and risk of endometriosis, or at least a window earlier in the disease process than at or after the time of surgical diagnosis. Moreover, our control selection from within the large NHSII cohort minimizes the bias typically introduced by prior case-control studies, which selected controls from women proven to be fertile or undergoing laparoscopy due to other pathology. The large sample size allows us to conduct subgroup analyses carefully, adjusting for potential confounders. Blood cohort participants were required to not have taken hormones within 6 months before the blood draw, which minimizes potential confounding by hormone replacement therapy exposure.

In addition, cases and controls were matched on infertility history, which minimizes the potential bias (e.g., confounding by indication) that may exist in prior case-control studies. However, there are potential caveats of matching on infertility. By matching on infertility, women with diseases that are highly associated with infertility (e.g., polycystic ovary syndrome) were oversampled. Although there is abundant literature suggesting insulin abnormality among polycystic ovary syndrome patients, there has been little evidence that polycystic ovary syndrome is also associated with abnormal IGF levels independent of BMI (23, 24). Matching (or stratifying) by infertility could be conditioning on the result of endometriosis (outcome) that can yield artificial findings (25–27). Although the prevalence of endometriosis is greater among women with infertility compared with those without, it remains unclear if this is due to causality or to diagnostic bias (28). Similar issues may also exist when adjusting for adulthood BMI, because BMI could be a result of IGF levels (29), and we could be conditioning on the result of the exposure. However, it is worth noting that adjusting for adulthood BMI made little difference in the multivariable results.

This study has several other limitations. It is possible that plasma IGF levels may not fully reflect peritoneal fluid levels and that peritoneal IGF levels may be a more proximal and therefore important predictor for endometriosis, given that peritoneal fluid is the microenvironment for endometrial implants in the peritoneal cavity as opposed to peripheral blood. It has been shown that IGF-1 levels in peritoneal fluid were approximately 60% of paired serum levels, and that IGFBP-3 in peritoneal fluid was approximately half of the serum concentrations (8), which are strong positive correlations. However, studying peritoneal IGF levels and risk of endometriosis prospectively is impractical, because it is difficult to obtain peritoneal fluid samples from healthy women through invasive procedure. The NHSII cohort does not have detailed information regarding the stage of endometriosis for all endometriosis cases. However, to our knowledge, among the 5 studies that investigated whether IGF levels differ by stage of disease (5, 11–14), only Gurgan et al. (5) found that the IGF-1 level was different between early and late stage of disease. There is great consensus that the existing revised American Society for Reproductive Medicine staging system does not correlate with presenting symptoms nor short- or long-term prognosis (30).

Another limitation of this study is that the mean age at diagnosis of endometriosis within this nested case-control population is 45 years, which is approximately 10 years older than the mean age of diagnosis in this cohort as a whole. This is attributable to the timing of the NHSII blood collection, which started its large-scale blood collection 7–9 years after baseline enrollment. Given that this prospective study design sought to quantify the association between IGF levels prior to endometriosis (i.e., endometriosis diagnosis), by definition women who had been diagnosed prior to blood collection were excluded as prevalent cases at "baseline" for the blood cohort. Consequently, the participants in this study were older than the average age of endometriosis in our cohort as a whole. This prospective study design with respect to age of incident diagnosis may reduce generalizability. However, to our knowledge, these are the only blood samples collected among women at risk for endometriosis with prospective follow-up. Because we saw elevated risks of endometriosis among women younger than 40 years at blood draw, the results may be more robust if the study population was younger. Certainly, a limitation also is the inability to identify the time of endometriosis onset at a molecular or cellular level, although this is true for all studies of endometriosis regardless of design or sampled populations. Finally, this study is a population-based case-control study, and it is likely that a proportion of the control group has asymptomatic disease or is symptomatic but has not reached diagnosis. However, as mentioned above, selecting controls from women who underwent tubal ligation or laparoscopy can introduce intractable bias. This potential misclassification of endometriosis cases is likely to be nondifferential because IGF-1 and IGFBP-3 levels are not associated with the misclassification, which could have biased the results toward the null. Moreover, given the low prevalence of undiagnosed endometriosis in the general population (<2% demonstrated by Zondervan et al. (31)), the impact on the results is likely to be minimal.

In summary, we found no association of plasma levels of IGF-1 or IGFBP-3 or of the IGF-1:IGFBP-3 molar ratio with endometriosis risk overall, where blood samples were collected, on average, 4 years prior to disease diagnosis. The elevated risks observed for all 3 biomarkers among relatively

younger women require confirmation in additional large prospective studies.

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APPENDIX

Insulin-Like Growth Factor Assays

As detailed in previous publications (32, 33), insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) were assayed by enzyme-linked immunosorbent assays with reagents provided by the Diagnostic Systems Laboratory (Webster, Texas). This methodology is more reproducible and appropriate for a large number of samples than the radioimmunoassay previously used (32). There were high correlations between the measurements from the enzyme-linked immunosorbent assays and radioimmunoassay for IGF-1 (Pearson's $r = 0.97$) (32) and IGFBP-3 (Spearman's $r = 0.85$) (33). Matched case-control triplets were shipped in the same batch and assayed together in a randomly determined order. Each batch included blinded, pooled quality-control samples to assess laboratory precision, and the within-batch coefficients of variation for all assays were <7% (mean = 6.3% for IGF-1 and mean = 4.1% for IGFBP-3). We found good within-person reproducibility of IGF-1 and IGFBP-3 within this cohort (34) over time (over 3 years, premenopausal women only, intraclass correlation coefficient (ICC) = 0.83 for IGF-1 and ICC = 0.76 for IGFBP-3). This level of reproducibility is similar to the levels of other biological variables, such as blood pressure and serum cholesterol measurements (ICC = 0.6–0.8 over several years), parameters considered reasonably well measured and consistent predictors of disease in epidemiologic studies.