

Insulin-like Growth Factor-I and Risk of High-Grade Cervical Intraepithelial Neoplasia

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Abstract

Insulin-like growth factors (IGF) and their binding proteins (IGFBP) have been implicated in the risk of several epithelial or glandular tumors, including prostate cancer, breast cancer, and colon cancer. Cervical cancer, which is also of epithelial origin, has been shown to overexpress receptors for IGF-I, and plasma levels of IGF-I have been positively associated with cervical cancer precursors in one epidemiologic study. In this case-control study, we investigated plasma levels of IGF-I and IGFBP-3 in relation to the risk of histologically confirmed high-grade cervical intraepithelial neoplasia (HGCIN) and the risk of human papillomavirus (HPV) infection. Included in this analysis were 329 cases and 621 controls recruited from clinics affiliated with two Montréal-area hospital centers. We observed a reduced risk of HGCIN for increasing levels of

IGF-I, with an adjusted odds ratio (OR) of 0.40 (95% confidence interval, 0.19-0.87) for the highest quartile relative to the lowest quartile of IGF-I. No association was observed between IGFBP-3 levels and HGCIN. Among controls, IGF-I was associated with a decreased risk of being positive for HPV-16 or HPV-18, with an adjusted odds ratio of 0.20 (95% confidence interval, 0.05-0.87) for the highest quartile relative to the lowest quartile of IGF-I. There was no association observed between IGFBP-3 levels and HPV infection status. IGF-I-mediated effects seemed to predominate among women <30 years of age. In contrast to the previously reported study, our results suggest that levels of IGF-I in young women may be inversely associated with HGCIN, a precursor to cervical cancer.

Introduction

Human papillomavirus (HPV) infection is now considered a necessary cause in the development of cervical cancer (1). However, only a small percentage of women who are infected with HPV infection go on to develop cervical cancer or its precursors (2). Current research strives to determine why certain HPV-positive women develop cervical cancer while others do not.

The insulin-like growth factor (IGF) family consists of IGF-I, IGF-II, and several IGF-binding proteins (IGFBP). Whereas IGF-II mainly plays a role as a key regulator in embryonic and fetal development (3), IGF-I continues to play a role throughout an individual's life. IGF-I is a broad-spectrum growth factor and has been shown to increase cell proliferation and transformation and to inhibit apoptosis (4), and may thus be involved in the progression to cancer. In *in vivo* and animal experiments, IGF-I has been associated with increased proliferation of human breast cancer cells (5) and decreased apoptosis (6) and has had a positive effect on tumor development in mice (6).

More than 90% of serum IGF-I is bound to IGFBP-3 (7), the most abundant of the six different IGFBPs identified. IGFBP-3 facilitates the transport of bound IGF-I to target tissues, regulates its interaction with the IGF-I receptor, and increases

its half-life by protecting it from degradation (7). IGFBP-3 also has an inhibitory effect on cell growth, which functions independent of IGF-I (8). In epidemiologic studies, positive associations between IGF-I levels and cancers of epithelial or glandular origin, including cancers of the breast (9-11), prostate (12-18), and colorectum (19-24), have been observed consistently. Conversely, the results of studies that have looked at the association between IGFBP-3 levels and cancer risk have been inconsistent with some studies showing inverse associations with cancers of the colorectum (19, 21) and lung (25, 26); other studies reporting positive associations with cancers of the prostate (15), breast (11), and colorectum (20); and other studies reporting null or nonsignificant associations (13, 14, 18, 22, 27).

Cervical cancer is also of epithelial origin; thus, elevated levels of IGF-I may enhance cervical carcinogenesis. There is evidence that the IGF-I receptor is overexpressed in cervical cancer cells, suggesting that they may be sensitive to IGF-I levels (28). Furthermore, one epidemiologic study has reported a strong positive association between high levels of IGF-I and both low-grade and high-grade squamous intraepithelial lesions (27). In the present study, we investigated the relationship between plasma levels of IGF-I and IGFBP-3 and the risk of high-grade cervical cancer precursors. In addition, we explored the role of IGF variables on HPV infection, independent of the risk of cervical cancer precursors, by examining the association of plasma levels of IGF-I and IGFBP-3 with HPV infection among the controls.

Materials and Methods

Study Design and Population. This case-control study was conducted as part of the Biomarkers of Cervical Cancer Risk

study, which has previously been described in detail (29). Cases were recruited from among women presenting at the colposcopy clinics of the collaborating hospitals of the McGill University Health Centre and the Centre Hospitalier de l'Université de Montréal for a suspected high-grade lesion. Women who were recruited as cases remained in the case series if they were histologically confirmed to have newly diagnosed high-grade cervical intraepithelial neoplasia (HGCIN), defined as cervical intraepithelial neoplasia grades 2 or 3, based on their enrollment biopsy. Controls were recruited during the same time period from among women presenting for their annual routine Pap smear test at family medicine and gynecology centers that referred women to the collaborating hospitals. To avoid outcome misclassification, women who were recruited as controls remained in the control series if their enrollment Pap test result was within normal limits or was consistent with benign cellular changes. A potential control was excluded if her cytologic result indicated any squamous abnormality. These women were eligible to be cases in the study if they attended one of the participating colposcopy clinics during the study period and met the case definition. Potential cases and controls were not enrolled if they met any of the following criteria: currently pregnant; had a history of high-grade cervical disease; had had removal of the cervix as a result of a hysterectomy or conization surgery; and a personal history of any cancer except nonmelanoma skin cancer.

From each participating woman, exfoliated cervical cells were collected using an Accelon biosampler (Medscand, Inc., Hollywood, FL) and resuspended in Preservcyt (Cytoc Corporation, Boxborough, MA). The cervical specimen was obtained before biopsy for cases and after the Pap test for controls and subsequently stored at 4°C until processing. In addition, a 10-mL blood sample was collected from consenting subjects by venipuncture in a heparinized Vacutainer tube. Blood samples were centrifuged at $1,500 \times g$ for 20 min. Plasma and buffy coat were aspirated and stored separately in individual Nunc vials at -70°C . Each woman also completed a self-administered questionnaire eliciting information on sociodemographic characteristics, smoking and alcohol consumption, reproductive and sexual history, and other risk factors for cervical neoplasia and HPV infection.

IGF Testing. Plasma levels of IGF-I were determined using ELISA (Diagnostics Systems Laboratory, Webster, TX) as previously described (12). Before the IGF-I assay, IGFBP was removed via acid-ethanol extraction. IGFBP-3 was also quantified using ELISA. Low- and high-concentration controls were included in each plate. Each sample was tested in duplicate and the mean was used for data analysis. If the relative difference between the two results exceeded 10%, the assay was repeated. For quality control, aliquots from a single pooled serum sample were randomly placed within each assay batch. Across all assay runs, the coefficients of variation for the low and high controls and for the serum pool were 3.7%, 5.6%, and 3.2% for IGF-I, and 8.9%, 3.0%, and 4.7% for IGFBP-3.

Cervical Specimen Processing and DNA Extraction. Cervical specimens were centrifuged at $13,000 \times g$ for 15 min at 22°C and resuspended in 300 μL of 20 mmol/L Tris buffer (pH 8.3). DNA was purified using the Master pure procedure. To determine specimen integrity, the presence of human β -globin DNA was tested for using the primers GH20 and PC04 (30). The extracted DNA was later used for HPV testing and typing.

HPV Testing and Typing. HPV DNA testing was done via a PCR protocol using the L1 consensus primers PGMY09/11 and typing using the reverse line blot assay as previously described (31). An extended line blot strip was used, which probed for 37 genital HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51,

52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39 (a subtype of HPV-82). Samples that were not positive for any of these types were considered HPV negative. Specimens that were negative for β -globin indicated a lack of sufficient DNA and were considered inadequate. Negative, weak positive, and strong positive controls were included in each amplification run.

Definition of Variables. IGF-I and IGFBP-3 were categorized into quartiles based on the distribution in control subjects, contextual to the type of analyses (i.e., whether it referred to all subjects or to strata defined by age). HPV types were classified as having either high-risk oncogenic potential or low-risk oncogenic potential (32). High-risk HPV types included types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. When HPV status was treated as a confounding or mediating variable, women were classified in mutually exclusive categories of HPV negative, positive for low-risk HPV types only, positive for at least one high-risk HPV type except HPV-16 and HPV-18, and positive for HPV-16 and/or HPV-18. We also examined HPV status as an outcome defined in three ways: those who tested positive for any HPV type; those who tested positive for at least one high-risk HPV type; and those who tested positive for HPV-16 and/or HPV-18, the HPV types most commonly found in cervical cancers.

Statistical Analysis. The correlation between IGF analytes and age was analyzed via least squares regression using linear and log-transformed values for the variables. The coefficient of determination (R^2) was used to determine the best fit. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated using logistic regression to measure the associations between each IGF variable with HGCIN and with each HPV status outcome among controls. All analyses were adjusted for or stratified by age, as appropriate. In addition, we adjusted for potential confounders or mediators selected from the list of variables in Table 1 if their inclusion in any of the models caused a change in the level-specific ORs for IGF-I or IGFBP-3 of $\geq 5\%$. Both IGF-I and IGFBP-3 were examined separately and also in a single model mutually adjusted for each other. We tested for trend by fitting models using the IGF variable treated as ordinal based on the median value for each quartile. In addition, using the Breslow-Day test, we assessed interaction between IGF-I and IGFBP-3, where each variable was dichotomized at the median. Based on the results, the interaction term for IGF-I and IGFBP-3 was not retained in any of the final models; thus, we chose to show only models with main effects.

Results

Between February 2001 and March 2005, 637 potential cases and 1,152 potential controls were approached to participate in this study. Sixty-six cases and 129 controls did not consent or refused to provide a blood specimen for a participation rate of 89.6% among cases and 88.8% among controls. Of the 571 eligible potential cases, 361 (62.3%) were confirmed to have HGCIN following their biopsy. The other potential cases had biopsy results indicating invasive disease; low-grade cervical intraepithelial neoplasia; other conditions such as cervicitis, squamous metaplasia, or parakeratosis; or a normal cervix, and thus did not meet the case definition. Of the 1,023 eligible potential controls, 911 (89.1%) had a Pap result within normal limits or indicating benign cellular changes. Because of the lag time between recruitment in our study and receiving the final biopsy or Pap test result, IGF-I and IGFBP-3 levels were assayed among the individuals that had their case or control status confirmed by pathology at the time that the samples were sent for testing, which included 329 cases and 621 eligible controls.

Table 1. Distribution of baseline characteristics by outcome status

Variable*	Cases (n = 329)	Controls (n = 621)
	n (%)	
Age, y		
<25	75 (22.8)	160 (25.8)
25-29	70 (21.3)	126 (20.3)
30-34	60 (18.2)	114 (18.4)
35-39	63 (19.2)	84 (13.5)
40-44	24 (7.3)	48 (7.7)
45-49	21 (6.4)	44 (7.1)
≥50	16 (4.9)	44 (7.1)
Ethnicity		
French Canadian	209 (63.5)	380 (61.2)
English Canadian	41 (12.5)	44 (7.1)
Other	79 (24.0)	197 (31.7)
Highest level of schooling		
Elementary	5 (1.5)	9 (1.5)
Secondary	86 (26.1)	134 (21.6)
College/undergraduate university	204 (62.0)	402 (64.7)
Graduate	31 (9.4)	75 (12.1)
Cigarette smoking		
Never	126 (38.3)	331 (53.3)
Former	72 (21.9)	121 (19.5)
Current	128 (38.9)	167 (26.9)
No. pregnancies		
0	123 (37.4)	282 (45.4)
1	71 (21.6)	111 (17.9)
2	55 (16.7)	103 (16.6)
≥3	77 (23.4)	122 (19.7)
Oral contraceptive use		
Never	31 (9.4)	63 (10.1)
Former	159 (48.3)	301 (48.5)
Current	135 (41.0)	246 (39.6)
Age at menarche, y		
8-11	63 (19.2)	117 (18.8)
12	98 (29.8)	184 (29.6)
13	80 (24.3)	155 (25.0)
≥14	85 (25.8)	159 (25.6)
Use of hormones to treat menopause/infertility		
Never	298 (90.6)	538 (86.6)
Ever	25 (7.6)	69 (11.1)
Age at first vaginal intercourse, y		
<16	105 (31.9)	137 (22.1)
16-17	93 (28.3)	187 (30.1)
18	48 (14.6)	102 (16.4)
≥19	69 (21.0)	171 (27.5)
Lifetime no. male vaginal sexual partners		
≤1	24 (7.3)	105 (16.9)
2-4	76 (23.1)	190 (30.6)
5-8	94 (28.6)	155 (25.0)
>8	129 (39.2)	163 (26.3)
HPV status		
HPV negative	12 (3.7)	437 (70.4)
Positive for any HPV type	312 (95.4)	184 (29.6)
Positive for only a low-risk HPV type	12 (3.7)	72 (11.6)
Positive for at least one high-risk HPV type	300 (91.7)	112 (18.0)
Positive for any high-risk HPV type except HPV-16/HPV-18	109 (33.3)	80 (12.9)

*Missing data are not listed. Total frequencies may slightly differ from the total number of cases and controls because of missing data for some variables.

The mean age (SD) was 32.3 (9.3) years for the cases and 32.4 (10.3) years for the controls. The majority of participants in this study identified themselves as French Canadian (Table 1). Cases were more likely to be current smokers, to have had a younger age at first vaginal sexual intercourse, and to have had a higher number of lifetime male vaginal sexual partners. Of HGCIN cases, 95.4% were positive for any HPV type, in contrast to only 29.6% of controls (Table 1). Positivity for high-risk types was higher in cases than in controls whereas positivity for low-risk types was higher in controls. Of the

HPV-positive cases diagnosed with HGCIN, 96.1% were positive for at least one high-risk HPV type and 34.9% were positive for HPV-16 or HPV-18. Among the HPV-positive controls, only 60.8% were positive for at least one high-risk HPV type and 43.5% positive for HPV-16 or HPV-18. The mean (SD) of IGF-I levels was 295.29 (99.46) ng/mL among cases and 317.15 (109.03) ng/mL among controls and that of IGFBP-3 levels was 5,122.89 (751.86) ng/mL among cases and 5,120.97 (784.71) ng/mL among controls.

IGF-I and IGFBP-3 levels were inversely correlated with age among both cases and controls (Fig. 1), the associations being stronger for IGF-I than for IGFBP-3. The coefficients of determination for the regression analysis using log-transformed values of both axes (best fit among four sets of regression comparisons) were 0.261 and 0.293 for IGF-I and 0.075 and 0.042 for IGFBP-3 among cases and controls, respectively. The *P* values (via *t*-statistic transformation) for all four coefficients were <0.00001.

Increasing IGF-I levels were inversely associated with the risk of HGCIN (Table 2). The OR for the highest versus the lowest quartile of IGF-I levels was 0.46 (95% CI, 0.30-0.72). When we additionally adjusted for ethnicity, sexual activity, IGFBP-3, and HPV status, the observed association was not appreciably different (multivariate OR, 0.40; 95% CI, 0.19-0.87; *P*_{trend} = 0.007). The inverse association did not change appreciably when the analysis was restricted to HPV-positive subjects (multivariate OR, 0.36; 95% CI, 0.18-0.71, for the highest versus the lowest quartile of IGF-I; *P*_{trend} = 0.001) and when restricted to high-risk HPV-positive participants (multivariate OR, 0.37; 95% CI, 0.15-0.91, for the highest versus the lowest quartile of IGF-I; *P*_{trend} = 0.010) with conservative covariate adjustment.

IGFBP-3 levels were not associated with the risk of HGCIN (Table 2). The multivariate-adjusted OR for the highest versus the lowest quartile was 1.15 (95% CI, 0.57-2.31; Table 2). Similarly, an association between IGFBP-3 and HGCIN was not observed when restricted to HPV-positive women (multivariate OR, 1.06; 95% CI, 0.56-1.99, for the highest versus the lowest quartile; *P*_{trend} = 0.85) or restricted to high-risk HPV-positive women (multivariate OR, 0.84; 95% CI, 0.36-0.99, for the highest versus the lowest quartile; *P*_{trend} = 0.84).

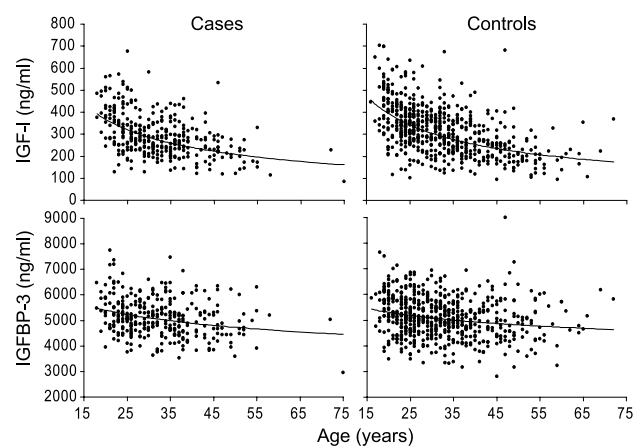


Figure 1. Scatter plots for the correlation of serum concentrations of IGF-I (top) and IGFBP-3 (bottom) with age, separately among cases (left) and controls (right). The curve in each graph represents the best-fit least-squares regression equation based on log-transformed values for both axes. The coefficients of determination (R^2) were 0.261 (IGF-I, cases), 0.293 (IGF-I, controls), 0.075 (IGFBP-3, cases), and 0.042 (IGFBP-3, controls). All four associated *P* values (via *t*-statistic transformation) were <0.00001.

Table 2. Associations between IGF variables and HGCIN in models controlling for different variables

IGF variable and levels	Cases, n (%)	Controls, n (%)	OR (95% CI)			P_{trend}^*
			Age adjusted [†]	Age, ethnicity, sexual activity, and mutual IGF-I and IGFBP-3 adjusted [‡]	Age, ethnicity, sexual activity, mutual IGF-I and IGFBP-3, and HPV status adjusted [§]	
IGF-I (quartiles), ng/mL						
Q1 (<239.0)	103 (31.31)	156 (25.12)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.007
Q2 (239.0-311.0)	110 (33.43)	155 (24.96)	0.92 (0.64-1.34)	0.90 (0.60-1.33)	0.89 (0.48-1.66)	
Q3 (311.0-382.0)	58 (17.63)	155 (24.96)	0.47 (0.31-0.71)	0.39 (0.25-0.62)	0.47 (0.23-0.94)	
Q4 (>382.0)	58 (17.63)	155 (24.96)	0.46 (0.30-0.72)	0.39 (0.24-0.65)	0.40 (0.19-0.87)	
IGFBP-3 (quartiles), ng/mL						
Q1 (<4,577.3)	76 (23.10)	155 (24.96)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.666
Q2 (4,577.3-5,088.8)	91 (27.66)	156 (25.12)	1.16 (0.79-1.70)	1.42 (0.95-2.14)	1.27 (0.67-2.40)	
Q3 (5,088.8-5,678.2)	93 (28.27)	155 (24.96)	1.21 (0.83-1.78)	1.60 (1.05-2.43)	1.57 (0.81-3.01)	
Q4 (>5,678.2)	69 (20.97)	155 (24.96)	0.93 (0.62-1.39)	1.40 (0.88-2.18)	1.15 (0.57-2.31)	

* P value for dose-response trend for the relation between IGF variable and outcome in the model adjusted for age, ethnicity, sexual activity, mutual IGF-I and IGFBP-3, and HPV status.

[†]Analyses adjusted for age group (<25, 25-29, 30-34, 35-39, 40-44, 45-49, and ≥ 50 y).

[‡]Analyses adjusted for age group, ethnicity (French Canadian, English Canadian, Other), sexual activity defined with age at first intercourse (<16, 16-17, 18, >19 y), number of sexual partners (1, 2-4, 5-8, and >8 partners), and mutual adjustment for IGF-I and IGFBP-3 according to the quartiles presented in the table.

[§]Analyses adjusted also for HPV status (negative, low-risk HPV only, high-risk HPV except HPV-16/HPV-18, and HPV-16/HPV-18).

When we examined the association between IGF-I and HGCIN stratified by the median age of cases (30 years), we observed that the inverse association was stronger among women ≤ 30 years of age but not apparent among women that were >30 years of age (Table 3). Similar to the results observed among all women, there was no association between IGFBP-3 and HGCIN among women that were ≤ 30 or >30 years of age (Table 3). Using in the age-stratified analyses the same cutoff points for quartile stratification defined among all control subjects (as shown in Table 2) produced comparable results (data not shown). Likewise, apart from the expected loss in precision, stratification into age tertiles produced similar results; the inverse correlation with IGF-I was evident for the first two tertiles (upper age bound, 35 years) but nonexistent among women ≥ 36 years of age (data not shown).

Among controls, levels of IGF-I and IGFBP-3 were not associated with the risk of being positive for any HPV type or positive for high-risk HPV types (Table 4). However, we observed an inverse association between IGF-I levels and positivity for HPV-16 or HPV-18 in the analysis that was adjusted for multiple covariates including IGFBP-3 (OR, 0.20; 95% CI, 0.05-0.87, for the highest versus the lowest quartile; $P_{\text{trend}} = 0.04$). There was no consistent pattern of association between IGFBP-3 and positivity for any of the HPV variables. Age-stratified analyses indicated that the above inverse correlation was maintained among women up to 30 years of age (OR, 0.18, for the highest versus the lowest quartile). However, due to the low HPV positivity among older control women, the OR estimates were very unstable and uninformative.

Discussion

The results from this study suggest that increasing levels of IGF-I are associated with a reduced risk of HGCIN. This inverse association was consistent whether examined among women overall or when restricted to participants that were HPV positive. However, the observed inverse association seemed to be stronger among younger women. We also observed that high IGF-I concentration was associated with a reduced risk of being positive for HPV-16 or HPV-18 among controls. Levels of IGFBP-3 were not associated with the risk of HGCIN or being HPV positive among controls. Because the concentration of IGF-I was strongly negatively correlated with age and HPV infection prevalence also decreases with age, it would have been possible that the negative association

between IGF-I and HGCIN risk could have been confounded by age. The prevalences of HPV-16/HPV-18 and other combined high-risk HPVs decreased monotonically with age among controls, but the same was observed only for HPV-16/HPV-18 among cases (data not shown). Despite our conservative approach to covariate adjustment and age stratification, IGF-I remained an independent explanatory variable for HPV infection with types 16 and/or 18 among controls and of HGCIN after controlling or restricting for HPV infection status, which suggests that IGF-I levels could mediate both upstream and downstream (relative to HPV infection) steps in cervical carcinogenesis, most notably among young women.

In vitro and animal studies have shown that IGF-I has a positive effect on cell proliferation and a negative effect on apoptosis (5, 6, 33, 34) and that IGF-I is correlated with tumor development (6, 35), which led to the hypothesis that increasing levels of IGF-I may lead to the development of cancer. The role of IGFBP-3 in cancer development is less clear because it plays several functions at the cellular level, which can affect cell proliferation both positively and negatively (36). Past studies have found both positive and inverse associations between IGFBP-3 and the risk of various types of cancer (37).

The results from most large prospective studies have indicated that increasing IGF-I levels are associated with an increased risk of cancer (reviewed in ref. 37). Relative risks indicating a positive association for the highest versus the lowest levels of IGF-I have ranged from 1.2 to 3.5 for cancers of the colorectum, prostate, breast, and lung, although null results and nonsignificant reduced risks of cancer have also been observed (37).

Mathur et al. (38, 39) found that serum IGF-II levels were elevated across the cervical lesion spectrum and proposed that this could be used as an aid for early diagnosis. In addition, two other epidemiologic studies have examined the relationship between IGF-I, IGFBP-3, and risk of cervical cancer precursors (27, 40). Wu et al. (27) observed a strong, significant increase in the risk of low-grade or high-grade squamous intraepithelial lesions for women with IGF-I serum levels in the highest versus the lowest quartiles (adjusted OR, 8.54; 95% CI, 4.15-17.60). On the other hand, IGFBP-3 was not associated with squamous intraepithelial lesion risk once adjusted for IGF-I levels (adjusted OR, 1.41; 95% CI, 0.74-2.61, for the highest versus the lowest quartile). In contrast, Serrano et al. (40) found results that were more in line with ours although they used controls ascertained to be HPV negative. These

authors found substantially reduced levels of IGF-I and molar ratios of IGF-I to IGFBP-3 among cancer cases than their age-matched controls (40).

Our findings of an inverse association between IGF-I levels and HGCIN are in contrast with the findings for other cancers. One reason may be that the natural history of cervical cancer differs from that of the other cancers, for which sex hormones play a large role (e.g., breast and prostate cancers). Alternatively, our study was limited to preinvasive squamous lesions. Because of their rarity, glandular precursor lesions were not studied. It is conceivable that a positive relationship may exist between the latter and IGF-I, in line with findings for cancer sites in which adenocarcinomas predominate, such as for breast and prostate.

However, this would not explain why our results differ from the study by Wu et al. (27) on cervical cancer precursors. In that study, the mean IGF-I and IGFBP-3 levels were lower than that observed in our study and the mean age of participants was ~10 years younger. In addition, the participants of the study by Wu et al. were drawn from an economically disadvantaged and predominantly minority U.S. population, whereas the majority of the participants of our study were of Caucasian ancestry and had a high level of education. Both ethnicity and socioeconomic status may influence IGF-I levels, the latter potentially influencing IGF-I levels through indirect ways such as diet and nutrition (41-43), and it is possible that these factors may modify the association between IGF-I and HGCIN.

It is unclear why increased levels of IGF-I would have a protective effect on the risk of cervical cancer precursors and that the protection would be stronger among younger women. However, given that IGF-I was negatively associated with being positive for HPV-16 and HPV-18, the two HPV types most commonly associated with cervical cancer, one possible mechanism is that IGF-I decreases a woman's risk of HGCIN by decreasing her risk of being positive for HPV-16 and/or HPV-18, perhaps via increased turnover of the cervical

epithelium, thus reducing the duration of infections. Alternatively, IGF-I mediation of immune mechanisms could counter proliferation of infected cells (44). Because IGF-I levels decline with age, the stronger association observed among younger women may reflect a greater effect of IGF-I on HPV-16 and HPV-18 positivity due to higher IGF-I levels overall, possibly countering establishment of persistent infections.

Because of the cross-sectional ascertainment of HPV status in this study, there was no way of knowing whether the HPV infections detected among controls were persistent or transient. However, if the infections among controls did in fact represent persistent infections, then the results for IGF-I and HPV-16/HPV-18 positivity among controls might suggest that IGF-I may somehow negatively affect HPV persistence, and possibly viral load, and in doing so reduces the risk of HGCIN. It is noteworthy, however, that the negative association between IGF-I and lesion risk was particularly noticeable in the HPV-restricted analyses.

Given the key causative role of HPV infection in cervical cancer, it is possible that IGF-I may not play as large a role in cervical cancer development as for other cancers. Because of the very small number of HPV negative cases ($n = 12$), we were unable to examine IGF-I in association with HGCIN among HPV-negative women. Furthermore, despite our findings using HPV-restricted analysis, it is not possible to know if IGF-I plays a positive role downstream of what we tested here (i.e., in the progression of HGCIN to cervical cancer). It also cannot be ruled out that the presence of HGCINs had an effect on IGF levels. Cohort investigations will be needed to assess the association independently of the possible influence that the disease process may have on IGF levels.

There are several limitations and sources of potential bias in this study. Ideally, IGF-I and IGFBP-3 would have been measured before the development of the outcome. Due to the cross-sectional nature of the primary variable in this case-control study, the possibility of the outcome having an effect on IGF-I and IGFBP-3 levels cannot be discounted. Although many of

Table 3. Adjusted associations between IGF variables and HGCIN stratified according to median age

IGF variable and levels	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR (95% CI)			<i>P</i> _{trend}
			Age-adjusted*	Age, ethnicity, sexual activity, and mutual IGF-I and IGFBP-3 adjusted [†]	Age, ethnicity, sexual activity, mutual IGF-I and IGFBP-3, and HPV status adjusted [‡]	
≤30 y (461 women)						
IGF-I (quartiles), ng/mL						
Q1 (<294.0)	66 (42.31)	77 (25.25)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.016
Q2 (294.0-353.0)	32 (20.51)	77 (25.25)	0.50 (0.29-0.84)	0.43 (0.24-0.79)	0.40 (0.16-0.96)	
Q3 (353.0-424.0)	27 (17.31)	75 (24.59)	0.42 (0.24-0.74)	0.33 (0.18-0.62)	0.35 (0.14-0.87)	
Q4 (>424.0)	31 (19.87)	76 (24.92)	0.48 (0.27-0.85)	0.36 (0.19-0.71)	0.31 (0.12-0.76)	
IGFBP-3 (quartiles), ng/mL						
Q1 (<4,714.8)	38 (24.36)	76 (24.92)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.729
Q2 (4,714.8-5,204.7)	45 (28.85)	76 (24.92)	1.19 (0.70-2.04)	1.40 (0.76-2.58)	1.41 (0.58-3.44)	
Q3 (5,204.7-5,787.0)	40 (25.64)	78 (25.57)	1.04 (0.60-1.80)	1.38 (0.73-2.59)	1.28 (0.50-3.27)	
Q4 (>5,787.0)	33 (21.15)	75 (24.59)	0.90 (0.51-1.61)	1.29 (0.64-2.58)	0.91 (0.34-2.42)	
>30 y (488 women)						
IGF-I (quartiles), ng/mL						
Q1 (<208.0)	46 (25.59)	82 (26.03)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.506
Q2 (208.0-261.0)	49 (28.32)	78 (24.76)	1.04 (0.67-1.74)	0.99 (0.57-1.72)	1.05 (0.40-2.74)	
Q3 (261.0-332.0)	50 (28.90)	77 (24.44)	1.06 (0.62-1.79)	0.94 (0.53-1.68)	1.72 (0.62-4.76)	
Q4 (>332.0)	28 (16.18)	78 (24.76)	0.55 (0.30-1.00)	0.47 (0.24-0.92)	0.59 (0.19-1.89)	
IGFBP-3 (quartiles), ng/mL						
Q1 (<4,496.7)	47 (27.17)	79 (25.08)	1.00 (reference)	1.00 (reference)	1.00 (reference)	0.486
Q2 (4,496.7-4,965.9)	40 (23.12)	78 (24.76)	0.83 (0.49-1.41)	0.89 (0.51-1.55)	0.58 (0.22-1.54)	
Q3 (4,965.9-5,461.9)	49 (28.32)	79 (25.08)	1.01 (0.60-1.69)	1.15 (0.66-2.02)	1.12 (0.40-3.11)	
Q4 (>5,461.9)	37 (21.39)	79 (25.08)	0.78 (0.46-1.34)	0.99 (0.55-1.80)	1.24 (0.44-3.48)	

*Analyses adjusted for age group (<22, 22-25-, 26-30) and (31-34, 35-40, 41+).

[†]Analyses also adjusted for ethnicity (French Canadian, English Canadian, Other), age at first intercourse (<16, 16-17, 18, >19 y), number of sexual partners (1, 2-4, 5-8, and >8 partners), and mutual adjustment for IGF-I and IGFBP-3 according to the age-specific quartiles in the table.

[‡]Analyses adjusted also for HPV status (negative, low-risk HPV only, high-risk HPV except HPV-16/HPV-18, and HPV-16/HPV-18).

Table 4. Adjusted association between IGF variables and HPV positivity among controls

IGF marker	Overall positivity		High-risk HPV types		HPV-16/HPV-18	
	OR (95% CI)	<i>P</i> _{trend}	OR (95% CI)	<i>P</i> _{trend}	OR (95% CI)	<i>P</i> _{trend}
Age adjusted*						
IGF-I (quartiles)						
Q1	1.00 (reference)	0.853	1.00 (reference)	0.990	1.00 (reference)	0.138
Q2	1.26 (0.72-2.20)		0.84 (0.42-1.68)		0.40 (0.13-1.26)	
Q3	0.88 (0.49-1.57)		0.68 (0.34-1.36)		0.43 (0.15-1.25)	
Q4	1.17 (0.65-2.10)		0.96 (0.48-1.92)		0.38 (0.12-1.13)	
IGFBP-3 (quartiles)						
Q1	1.00 (reference)	0.403	1.00 (reference)	0.252	1.00 (reference)	0.883
Q2	1.18 (0.70-2.00)		1.75 (0.90-3.39)		1.81 (0.63-5.19)	
Q3	1.08 (0.64-1.83)		1.28 (0.65-2.51)		0.72 (0.21-2.47)	
Q4	1.29 (0.77-2.16)		1.68 (0.87-3.23)		1.38 (0.47-4.03)	
Adjusted for age and additional empirical and a priori confounders [†]						
IGF-I (quartiles)						
Q1	1.00 (reference)	0.631	1.00 (reference)	0.607	1.00 (reference)	0.044
Q2	1.45 (0.76-2.79)		0.86 (0.38-1.95)		0.39 (0.09-1.53)	
Q3	0.75 (0.37-1.52)		0.49 (0.21-1.18)		0.22 (0.06-0.90)	
Q4	1.02 (0.48-2.13)		0.78 (0.32-1.93)		0.20 (0.05-0.87)	
IGFBP-3 (quartiles)						
Q1	1.00 (reference)	0.347	1.00 (reference)	0.289	1.00 (reference)	0.555
Q2	1.22 (0.65-2.27)		1.89 (0.87-4.10)		2.74 (0.80-9.37)	
Q3	1.15 (0.60-2.18)		1.40 (0.62-3.19)		1.08 (0.25-7.54)	
Q4	1.40 (0.73-2.66)		1.81 (0.80-4.11)		1.96 (0.51-7.54)	

*Analyses adjusted for age group (<25, 25-29, 30-34, 35-39, 40-44, 45-49, and ≥50 y).

[†]Analyses adjusted for age group (as above), ethnicity (French Canadian, English Canadian, Other), age at first intercourse (<16, 16-17, 18, >19 y), number of sexual partners (1, 2-4, 5-8, and >8 partners), cigarette smoking (current, former, never), oral contraceptive use (never, ever), use of hormones to treat menopause/infertility (never, ever), age at menarche (<12, 12, 13, 14+ years), and mutual adjustment for IGF-I and IGFBP-3 according to the quartiles among controls (same cutoff points as in Table 2).

the factors suspected to be associated with IGF-I and IGFBP-3 levels were measured, including age and sexual activity, residual confounding may have occurred if these factors were not measured accurately enough. Other determinants postulated to be associated with IGF-I and/or IGFBP-3 were not measured. Some studies have found a relationship between IGF variables and body mass index (32-35), diet (41, 45), and physical activity (36). Any confounder associated with IGF variables that differed between cases and controls that was not measured could have led to bias. Variables related to energy balance and caloric intake should be considered in future investigations.

As well, any study that relies on voluntary participation may lead to self-selection, which may affect the generalizability of the results. Women who provided a blood sample for this study and those who did not were compared on their sociodemographic characteristics; they did not differ significantly on any of the characteristics examined. Data on those who refused to both fill out a questionnaire and give a blood sample were not available, but given the small number of women who refused to participate, it is unlikely this was a major source of bias.

Misclassification could also have occurred at the level of the outcome, exposure, or confounders. Case status was confirmed by cervical biopsy, whereas controls were confirmed by cytology only. Given that cases were more rigorously examined, it is more likely that misclassification could have occurred among controls. There may have been a number of false negatives among controls, which may have led to a misrepresentation of the OR. To avoid misclassification with respect to IGF-I and IGFBP-3 levels, each sample was tested twice and tested in as few batches as possible by the same laboratory technician. Lastly, because most confounders were self-reported, some misclassification with respect to confounders is certain but is unlikely to have created much bias in the final ORs.

On the other hand, there were many strengths to this study. HGCINs are a known precursor to the development of cervical

cancer, and cervical cancer is very rare in this population. By using HGCINs as the outcome, it allowed us to measure the potential effect of IGF-I and IGFBP-3 levels on the development of cervical cancer while lessening the concern that a serious condition such as cancer would have affected IGF-I and IGFBP-3 levels, an argument that counters the possible reverse causality concern expressed above. In this way, the IGF levels should reflect as accurately as possible precancer levels. This study also had a very high participation rate, lessening the chance of selection bias. Controls were drawn from a similar population as the cases, and controls that later on were determined to have HGCIN were eligible to be cases. Much care was taken to ensure the accuracy of the measurement of the exposure, and laboratory personnel were blinded to case-control status.

There is very little research on the relationship between IGF variables and cervical cancer and its precursors. Because of the contrasting results obtained in this study as compared with that by Wu et al. (27), further studies are warranted to elucidate the relationship between IGF-I, IGFBP-3, HPV infection, and HGCINs, particularly cohort investigations. The relationships between IGF-I levels and HGCIN and HPV-16 and HPV-18 infections observed in this study are much stronger than for many currently accepted risk factors for cancer, and thus should be explored. Not only could this information help to better understand the natural history of cervical cancer and why some HPV-positive women progress to disease and others do not but IGF variables could also potentially serve as biomarkers of disease progression.

Appendix A. Members of the Biomarkers of Cervical Cancer Risk Study Team

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References

- Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;189:12–9.
- Franco EL, Harper DM. Vaccination against human papillomavirus infection: a new paradigm in cervical cancer control. *Vaccine* 2005;23:2388–94.
- Lighten AD, Hardy K, Winston RM, Moore GE. Expression of mRNA for the insulin-like growth factors and their receptors in human preimplantation embryos. *Mol Reprod Dev* 1997;47:134–9.
- Macaulay VM. Insulin-like growth factors and cancer. *Br J Cancer* 1992;65:311–20.
- Yang X-F, Beamer WG, Huynh H, Pollak M. Reduced growth of human breast cancer xenografts in hosts homozygous for the IGF1 mutation. *Cancer Res* 1996;56:1509–11.
- Dunn SE, Kari FW, French J, et al. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res* 1997;57:4667–72.
- Jones JL, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* 1995;16:3–34.
- Rajah R, Valentinis B, Cohen P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor β 1 on programmed cell death through a p53- and IGF-independent mechanism. *J Biol Chem* 1997;272:12181–8.
- Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor I and risk of breast cancer. *Lancet* 1998;351:1393–6.
- Bohlik K, Cramer DW, Tichopoulos D, Mantzoros CS. Insulin-like growth factor-I in relation to premenopausal ductal carcinoma *in situ* of the breast. *Epidemiology* 1998;9:570–1.
- Yu H, Jin F, Shu XO, et al. Insulin-like growth factors and breast cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev* 2002;11:705–12.
- Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* 1998;279:563–6.
- Chokkalingam AN, Pollak M, Fillmore C-M, et al. Insulin-like growth factors and prostate cancer: a population-based case-control study in China. *Cancer Epidemiol Biomarkers Prev* 2001;10:421–7.
- Harman SM, Metter EJ, Blackman MR, Landis PK, Carter HB. Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. *J Clin Endocrinol Metab* 2000;85:4528–65.
- Li L, Yu H, Schumacher F, Casey G, Witte JS. Relation of serum insulin-like growth factor-I (IGF-I) and IGF binding protein-3 to risk of prostate cancer (United States). *Cancer Causes Control* 2003;14:721–6.
- Oliver SE, Gunnell D, Donovan J, et al. Screen-detected prostate cancer and the insulin-like growth factor axis: results of a population-based case-control study. *Int J Cancer* 2004;108:887–2.
- Stattin P, Bylund A, Rinaldi S, et al. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. *J Natl Cancer Inst* 2000;92:1910–7.
- Wolk A, Mantzoros CS, Andersson S-O, et al. Insulin-like growth factor I and prostate cancer risk: a population-based case-control study. *J Natl Cancer Inst* 1998;90:911–5.
- Giovannucci E, Pollak MN, Platz EA, et al. A prospective study of plasma insulin-like growth factor-I and binding protein-3 and risk of colorectal neoplasia in women. *Cancer Epidemiol Biomarkers Prev* 2000;9:345–9.
- Kaaks R, Toniolo P, Akhmedkhanov A, et al. 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.
- Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst* 1999;91:620–5.
- Nomura AM, Stemmermann GN, Lee J, Pollak MN. Serum insulin-like growth factor I and subsequent risk of colorectal cancer among Japanese-American men. *Am J Epidemiol* 2003;158:424–31.
- Palmqvist R, Hallmans G, Rinaldi S, et al. Plasma insulin-like growth factor I, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden. *Gut* 2002;50:642–6.
- Probst-Hench NM, Yuan J-M, Stanczyk FZ, Gao Y-T, Ross RK, Yu MC. IGF-I, IGF-2 and IGFBP-3 in prediagnostic serum: association with colorectal cancer in a cohort of Chinese men in Shanghai. *Br J Cancer* 2001;85:1695–9.
- London SJ, Yuan J-M, Travlos GS, et al. Insulin-like growth factor I, IGF-binding protein 3, and lung cancer risk in a prospective study of men in China. *J Natl Cancer Inst* 2002;94:749–54.
- Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X. Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. *J Natl Cancer Inst* 1999;91:151–6.
- Wu X, Tortolero-Luna G, Zhao H, Phatak D, Spitz MR, Follen M. Serum levels of insulin-like growth factor I and risk of squamous intraepithelial lesions of the cervix. *Clin Cancer Res* 2003;9:3356–61.
- Steller MA, Delgado CH, Zou Z. Insulin-like growth factor II mediates epidermal growth factor-induced mitogenesis in cervical cancer cells. *Proc Natl Acad U S A* 1995;92:11970–4.
- Koushik A, Ghosh A, Duarte-Franco E, et al. The p53 codon 72 polymorphism and risk of high-grade cervical intraepithelial neoplasia. *Cancer Detect Prev* 2005;29:307–16.
- Coutlee F, Rouleau D, Petignat P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGM1 primers and the linear array HPV genotyping test. *J Clin Microbiol* 2006;44:1998–2006.
- Coutlee F, Gravitt P, Kornegay J, et al. Use of PGM1 primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol* 2002;40:902–7.
- Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27.
- Furlanetto RW, Harwell SE, Frick KK. Insulin-like growth factor-I induces cyclin D1 expression in MG63 human osteosarcoma cells *in vitro*. *Mol Endocrinol* 1994;8:510–7.
- Sell C, Baserga R, Rubin R. Insulin-like growth factor I (IGF-I) and the IGF-I receptor prevent etoposide-induced apoptosis. *Cancer Res* 1995;55:303–6.
- Berrigan D, Perkins SN, Haines DC, Hursting SD. Adult-onset calorie-restriction and fasting delay spontaneous tumorigenesis in p53-deficient mice. *Carcinogenesis* 2002;23:817–22.
- Voskuil DW, Bueno de Mesquita HB, Kaaks R, et al. Determinants of circulating insulin-like growth factor (IGF)-I and IGF binding proteins 1-3 in premenopausal women: physical activity and anthropometry (Netherlands). *Cancer Causes Control* 2001;12:951–8.
- Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nat Rev Cancer* 2004;4:505–18.
- Mathur SP, Mathur RS, Underwood PB, Kohler MF, Creasman WT. Circulating levels of insulin-like growth factor-II and IGF-binding protein 3 in cervical cancer. *Gynecol Oncol* 2003;91:486–93.
- Mathur SP, Mathur RS, Gray EA, et al. Serum vascular endothelial growth factor C (VEGF-C) as a specific biomarker for advanced cervical cancer: Relationship to insulin-like growth factor II (IGF-II), IGF binding protein 3 (IGF-BP3) and VEGF-A. *Gynecol Oncol* 2005;98:467–83.
- Serrano ML, Romero A, Cendales R, Sanchez-Gomez M, Bravo MM. Serum levels of insulin-like growth factor-I and -II and insulin-like growth factor binding protein 3 in women with squamous intraepithelial lesions and cervical cancer. *Biomedica* 2006;26:258–68.
- Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev* 2002;11:852–61.
- DeLellis K, Ingles S, Kolonel L, et al. IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. *Br J Cancer* 2003;88:277–82.
- Jernstrom H, Chu W, Vesprini D, et al. Genetic factors related to racial variation in plasma levels of insulin-like growth factor-1: implications for premenopausal breast cancer risk. *Mol Genet Metab* 2001;72:144–54.
- Taguchi T, Takenouchi H, Matsui J, et al. Involvement of insulin-like growth factor-I and insulin-like growth factor binding proteins in pro-B-cell development. *Exp Hematol* 2006;34:508–18.
- Kaklamani VG, Linos A, Kaklamani E, Markaki I, Koumantaki Y, Mantzoros CS. Dietary fat and carbohydrates are independently associated with circulating insulin-like growth factor 1 and insulin-like growth factor binding protein 3 concentrations in healthy adults. *J Clin Oncol* 1999;17:3291–8.