Insulin, Insulin-like Growth Factor-I, Endogenous Estradiol, and Risk of Colorectal Cancer in Postmenopausal Women

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Abstract

Obesity is a risk factor for colorectal cancer, and hyperinsulinemia, a common condition in obese patients, may underlie this relationship. Insulin, in addition to its metabolic effects, has promitotic and antiapoptotic activity that may be tumorigenic. Insulin-like growth factor (IGF)-I, a related hormone, shares sequence homology with insulin, and has even stronger mitogenic effects. However, few prospective colorectal cancer studies directly measured fasting insulin, and none evaluated free IGF-I, or endogenous estradiol, a potential cofactor in postmenopausal women. Therefore, we conducted a case-cohort investigation of colorectal cancer among nondiabetic subjects enrolled in the Women's Health Initiative Observational Study, a prospective cohort of 93,676 postmenopausal women. Fasting baseline serum specimens from all incident colorectal cancer cases (n = 438) and a random subcohort (n = 816) of Women's Health Initiative Observational Study subjects were tested for insulin, glucose, total IGF-I, free IGF-I, IGF binding protein-3, and estradiol. Comparing extreme quartiles, insulin [hazard ratio (HR)$_{q4-q1}$, 1.73; 95% confidence interval (CI), 1.16–2.57; P_{trend} = 0.005], waist circumference (HR$_{q4-q1}$, 1.82; 95% CI, 1.22–2.70; P_{trend} = 0.001), and free IGF-I (HR$_{q4-q1}$, 1.35; 95% CI, 0.92–1.98; P_{trend} = 0.05) were each associated with colorectal cancer incidence in multivariate models. However, these associations each became nonsignificant when adjusted for one another. Endogenous estradiol levels, in contrast, were positively associated with risk of colorectal cancer (HR comparing high versus low levels, 1.53; 95% CI, 1.05–2.22), even after control for insulin, free IGF-I, and waist circumference. These data suggest the existence of at least two independent biological pathways that are related to colorectal cancer: one that involves endogenous estradiol, and a second pathway broadly associated with obesity, hyperinsulinemia, and free IGF-I. [Cancer Res 2008;68(1):329–37]

Introduction

Colorectal cancer is the third most common malignancy among both women and men in the U.S., with an incidence of 52 cases per 100,000 person-years (a total of 154,000 cases each year).[7] The risk of colorectal cancer is increased in obese patients—in the range of 20% to 50% higher than in normal weight individuals (1)—and with one third of adults from the U.S. now considered to be obese, this relationship may be an important and potentially growing source of colorectal cancer cases. However, the biological factors that underlie colorectal cancer's relation with obesity are not well understood. Determining these factors would provide insight into the mechanisms of colorectal tumorigenesis and, once known, these factors could have clinical utility, either as biomarkers (e.g., to identify patients at high risk of colorectal cancer), and/or as molecular targets for the development of interventions to prevent or treat colorectal tumors.

Hyperinsulinemia has long been hypothesized to play a role in the obesity-colorectal cancer relationship (2, 3). Insulin resistance and hyperinsulinemia are prevalent in obese patients and insulin, in addition to its metabolic effects, has promitotic and antiapoptotic activity that may be tumorigenic. In laboratory models, for example, high insulin levels have been shown to promote the development of aberrant crypt foci in the colon (which are posited to be colorectal cancer precursors), as well as the growth of colon cancer cells (4). Furthermore, overexpression of the insulin receptor (IR) can induce cell transformation *in vitro* (5), and human colorectal adenocarcinomas have been shown to express the IR at high levels, indicating that these cells may be sensitive to the growth effects of insulin (6).

Few prospective studies, however, have directly assessed the relation of hyperinsulinemia with colorectal cancer, and their results have been conflicting. Of the four studies we are aware of (7–10), two reported positive associations between hyperinsulinemia and colorectal cancer (7, 10), but only one of these conducted multivariate analyses and the association was no longer statistically significant following adjustment for other risk factors (10). The remaining two studies found no association between insulin and colorectal cancer, but measured insulin using nonfasting blood specimens, which complicates their interpretation (8, 9). The unavailability of fasting serum samples may have also been the reason that other prospective investigations indirectly assessed the relation of insulin with colorectal cancer by measuring levels of C-peptide, a marker of insulin secretion. C-peptide is often used in such settings because C-peptide levels are thought to be less variable than insulin. C-peptide levels do, however, substantially increase postprandially (11), and there is an imperfect correlation of C-peptide and insulin even when measured in fasting specimens (12). Therefore, although recent prospective studies of C-peptide have provided important data, their findings have been

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[7] SEER Database. Available from: http://seer.cancer.gov/.

inconsistent (13–17), and direct measurement of insulin, the putative carcinogenic agent, may provide a more accurate estimation of the relation of hyperinsulinemia with colorectal cancer.

A related peptide hormone, insulin-like growth factor (IGF)-I, shares 40% amino acid sequence homology and downstream signaling pathways with insulin, but has much stronger mitogenic and antiapoptotic activity than insulin. At least four prospective investigations reported a significant positive association of colorectal cancer with circulating IGF-I levels (15, 18–20), and two of these studies also observed a significant inverse association with levels of IGF binding protein-3 (IGFBP-3), the most abundant IGF binding protein in circulation (19, 20). Other studies though, reported conflicting results, observing no relationship between IGF-I and colorectal cancer (13, 16, 21), as well as either positive (13, 18) or null associations with IGFBP-3 (16, 21). No prior studies, however, measured free (unbound) IGF-I levels, which has been hypothesized to be the main bioactive component of IGF-I in circulation (22).

Lastly, we note that no studies have ever examined the relation of endogenous estrogen levels with colorectal cancer, even though obesity is associated with high serum estradiol, and the use of oral hormone therapy (HT) has been related to reduced risk of colorectal cancer (23). Interestingly, although several laboratory studies reported that estrogen has antitumorigenic activity in keeping with the protective effects of hormone therapy (24–33), other studies found that estrogen has mitogenic effects on colorectal cells (34–42). Furthermore, oral estrogens have certain biological effects that differ from those of endogenous estrogens; most notably, oral hormone therapy exposes the liver to a large bolus of estrogen altering hepatic protein synthesis, a phenomenon known as the "first-pass effect" (43). Endogenous estrogen exposure, therefore, warrants separate study as a risk factor for colorectal cancer. In any event, it may be important to control for estradiol levels in studies of colorectal cancer and the insulin/IGF axes because there is biological cross-talk between the sex hormone and insulin/IGF pathways.

The current study, therefore, assessed the associations of incident colorectal cancer with hyperinsulinemia, IGF-I, and estradiol, as well as the degree to which these factors might account for the obesity-colorectal cancer relationship. We note that this study was specifically designed to have adequate statistical power to concurrently assess these moderately correlated variables.

Materials and Methods

Study Population

The Women's Health Initiative. The observational arm of the Women's Health Initiative Observational Study (WHI-OS) is a longitudinal cohort of 93,676 postmenopausal women ages 50 to 79 years who were recruited at 40 different clinical centers across the United States between October 1, 1993 and December 31, 1998 (44). At baseline, women provided informed consent and completed questionnaires regarding demographic and behavioral factors, medical history, and use of medications (including hormone therapy). A physical examination was conducted that included waist, hip, height and weight measurements. Serum samples were obtained following an overnight fast of at least 8 h, and were immediately centrifuged and stored at −70°C.[8] Cancer outcomes were initially ascertained through annual self-administered questionnaires, then case status and detailed diagnosis were formally determined through centralized review of all pathology reports, discharge and consultant summaries, operative and radiology reports, and tumor registry abstracts. Cases were coded according to National Cancer Institute Surveillance, Epidemiology and End-Results guidelines (45). As of February 29, 2004, the date when the subjects of this colorectal cancer case-cohort study were selected, there was a mean follow-up time of 77 months, 1.6% of the women had been lost to follow-up and 4.7% were deceased.

Study subjects. A case of incident colorectal cancer was defined as the diagnosis of disease (International Classification of Diseases for Oncology site codes 153.0–153.4, 153.6–153.9, and 154.0–154.1) after >1 year of follow-up (lag time) in a nondiabetic individual with no history of colorectal cancer at baseline. All cases of incident colorectal cancer who met the above criteria (n = 438), except two with missing data, were included in our study. A subcohort of 816 subjects randomly selected from all women in the WHI-OS at baseline and who met the same inclusion and exclusion criteria as the cases was used as the comparison group. Diabetics were excluded, as in several previous studies of the insulin/IGF-axis because of the uncertain effects an abnormal hormonal milieu might have on the relation of these factors with cancer.

Laboratory Methods

Serum insulin and glucose were measured by the designated central laboratory for WHI, Medical Research Laboratories, Highland Heights, KY, and insulin resistance was estimated using the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index ([fasting insulin (μIU/mL) × fasting glucose (mg/dL)] / 22.5; ref. 46). Serum estradiol levels were measured using the Vitros-Eci Immunodiagnostic Assay (Ortho-Clinical Diagnostics) at the Esoterix Center for Clinical Trials, the central laboratory designated by WHI for sex hormone assays. Concentrations of total IGF-I, free IGF-I, and IGFBP-3 were determined using commercially obtained ELISA (Diagnostic Systems Laboratories). Of note, "free IGF-I" testing by ELISA, unlike with ultrafiltration, measures not only the fraction of IGF-I that is unbound to IGFBPs but also "easily dissociable IGF-I", although these levels are well correlated (47). All estradiol, total IGF-I, free IGF-I, and IGFBP-3 tests were conducted in duplicate and the mean value for each duplicate pair was used as the result for analysis. Total IGF-I and IGFBP-3 tests with coefficients of variation (CV) >10% were repeated. For free IGF-I, a CV of >20% was used as the threshold because free IGF-I levels are low and, as mean values for a variable approach zero, the CV becomes mathematically sensitive to small changes in standard deviation. The Esoterix laboratory maintained its own quality control operations and any tests with CV >20% were repeated. Individual runs of any assay with quality control values outside of the expected range were repeated. Approximately 5% of the WHI-OS samples were retested in a blinded fashion. The correlations of assay values determined in the replicates were very high (total IGF-I, R^2 = 0.964; free IGF-I, R^2 = 0.903; IGFBP-3, R^2 = 0.895; insulin, R^2 = 0.984; glucose, R^2 = 0.947; estradiol, R^2 = 0.996). The estradiol assays were completed in a single batch. All other assays were completed in two separate batches. We tested the statistical equivalence of the age-adjusted hazard ratio (HR) estimates for the association of our serum measures and colorectal cancer between batches, and confirmed that the results did not significantly differ.

Statistical Analysis

As part of preliminary data analysis, the distributions of baseline characteristics among the cases and the subcohort (limited to those who did not later become cases) were compared and contrasted, with the age-adjusted Wilcoxon rank sum test (for continuous data) or Pearson's χ^2 (for categorical data) used to assess the statistical significance of any differences. All serologic data, in keeping with prior studies of insulin/IGF and cancer, were a priori expressed as quartiles or tertiles based on the distribution of results in the subcohort. For those assays conducted in two separate batches the quartiles were determined separately for each batch; an approach that minimized the possibility that even unrecognized variations in laboratory results across batch might affect our findings. Correlations between these categorical serologic data, age, and waist circumference were assessed using Spearman's correlation coefficient. To assess the effects of hormone therapy on each of the measured serologic factors, we determined their mean values by hormone therapy stratum

[8] Available from: http://www.whiscience.org/about/about_biospecimen.php.

categorized as (*a*) unopposed estrogen, (*b*) combined estrogen and progesterone, and (*c*) non–estrogen/estrogen + progesterone users, and compared these values using an ANOVA test.

In the main analysis, we estimated HRs for the associations of each serologic variable, as well as other risk factors, with risk of incident colorectal cancer using multivariate Cox proportional hazard regression models that employed the Self-Prentice method (48) for robust standard error estimates (to account for the case-cohort design), with time from enrollment as the underlying time scale. All models were adjusted for a priori determined established colorectal cancer risk factors, including: age categorized as (*a*) 50 to 54 years of age (referent), (*b*) 55 to 59, (*c*) 60 to 64, (*d*) 65 to 69, (*e*) 70 to 74, (*f*) 75 to 79; smoking categorized as (*a*) never (referent), (*b*) former, and (*c*) current; race/ethnicity categorized as (*a*) white (referent), (*b*) black, (*c*) Hispanic, and (*d*) other; physical activity assessed as metabolic equivalent tasks per hour per week (MET); waist circumference, categorized as (*a*) <75.0 cm, (*b*) 75.0 to <83.5 cm, (*c*) 83.5 to <93.0 cm, and (*d*) ≥93.0 cm; use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the preceding year, alcohol consumption, assessed as number of servings per week and categorized as (*a*) none (referent), (*b*) <3, and (*c*) ≥3; and family history of colorectal cancer. Additional variables, including colorectal cancer screening history and intake of calcium, folate, vitamin D, and fiber were evaluated as potential covariates but their inclusion in our a priori determined multivariate models did not alter the overall risk estimates and are not further discussed. Endogenous estradiol data were assessed only in women who did not use estrogen or estrogen + progesterone because standard assays cannot accurately measure the equine hormones present in most hormone therapy preparations. This created five nonoverlapping groups, i.e., non–HT users with (*a*) low (referent), (*b*) moderate, or (*c*) high estradiol tertile levels, (*d*) estrogen users, (*e*) estrogen + progesterone users. These groups were then parameterized as separate dummy variables with low estradiol as the common referent, which permitted potentially different effects of estrogen use, estrogen + progesterone use, and high estradiol (among non–estrogen/estrogen + progesterone users) to be modeled in a single model.

Analyses stratified by HT use (nonuser, estrogen use, or estrogen + progesterone use), waist circumference (≤median or >median), tumor location (colon versus rectum; distal versus proximal colon), and tumor stage (≤stage 2 and >stage 2) were also performed.

Results

Preliminary data analysis. Table 1 shows the characteristics of the study population at baseline. Cases were slightly older, had a higher weight, greater waist circumference, greater body mass index (kg/m^2), greater waist-to-hip ratio, and reported less physical activity than women in the subcohort (excluding those who later become cases). The colorectal cancer cases were also significantly more likely to be current or previous smokers and were less likely to currently be using estrogen + progesterone, whereas there was no difference in the use of estrogen. Almost all hormone therapy involved oral and not transdermal medication.

Correlations between each of the serologic factors as well as age and waist circumference were estimated using Spearman's correlation coefficient (Table 2). Briefly, total IGF-I had a moderate correlation with IGFBP-3 and free IGF-I, whereas insulin was strongly correlated with waist circumference and weakly correlated with free IGF-I and IGFBP-3. In contrast, endogenous estradiol had a moderate, positive correlation with waist circumference and insulin, and was inversely correlated with free IGF-I. However, only the correlation of fasting insulin and HOMA-IR index (a calculated measure of insulin resistance based on fasting insulin and glucose levels; see Laboratory Methods) was of sufficient strength to raise concerns regarding colinearity (which we defined as a correlation ≥0.85).

We also examined the effects of hormone therapy use on levels of insulin, IGF-I, and IGFBP-3. Mean insulin levels were 7.1 μIU/mL in women not using hormone therapy, but were significantly lower in women using estrogen (6.0 μIU/mL) and estrogen + progesterone (5.7 μIU/mL; $P_{ANOVA} < 0.0001$). Likewise, total IGF-I, free IGF-I, and IGFBP-3 levels varied by use of hormone therapy. Based on prior reports of the first-pass effect, the highest levels for these factors were expected to be found in women not using hormone therapy, and the lowest to be in those using estrogen (because progestins partly offset the oral estrogen effects). The current data were in keeping with these expectations. For free IGF-I, for example, levels were 0.47 ng/mL in nonusers, 0.32 ng/mL in women using estrogen, and 0.40 ng/mL in women using estrogen + progesterone ($P_{ANOVA} < 0.001$). There were not enough women reporting transdermal hormone therapy use to study separately, but their exclusion from the analysis did not change the above findings.

Risk of colorectal cancer. Table 3 shows the associations of incident colorectal cancer with each of the serologic variables and with obesity, adjusted only for age. Waist circumference was a stronger predictor of colorectal cancer incidence [HR for highest versus lowest quartile (HR_{q4-q1}), 2.04; 95% confidence interval (95% CI), 1.43–2.92; $P_{trend} = 0.0001$] than BMI or waist-to-hip ratio, and was used as the primary measure of obesity in all subsequent multivariable models. The other factors associated with colorectal cancer included insulin, HOMA-IR index, and endogenous estradiol. The results for estradiol suggested a threshold effect; that is, the HRs for the second (HR, 1.67; 1.14–2.46) and third (HR, 1.43; 0.95–2.16) estradiol tertiles, compared with the first tertile, were similar and of statistical significance and borderline statistical significance, respectively. As a single joint variable, the upper two tertiles of estradiol were associated with a 1.57-fold (95% CI, 1.10–2.22) increased risk of incident colorectal cancer relative to levels in the first tertile. In contrast, total IGF-I, IGFBP-3, and glucose were not associated with risk of colorectal cancer in age-adjusted or more comprehensive multivariate analyses (data not shown); this included models that concurrently adjusted for both IGF-I and IGFBP-3, or assessed the IGF-I/IGFBP-3 molar ratio. Only free IGF-I had a more significant effect in multivariate models than in simple age-adjusted models (as shown below).

Table 4 shows multivariate results adjusted for established colorectal cancer risk factors including age, smoking, ethnicity, family history of colorectal cancer, physical activity, use of NSAIDs, and alcohol consumption (Table 4). Waist circumference remained significantly associated with risk of colorectal cancer in these adjusted models (HR_{q4-q1}, 1.82; 95% CI, 1.22–2.70; $P_{trend} = 0.001$), and this association was largely unaltered by additional adjustment for use of hormone therapy and endogenous estradiol levels in women not using hormone therapy (HT/estradiol; see Statistical Analysis). Adjustment for free IGF-I also had no effect. However, the association between waist circumference and colorectal cancer was attenuated and lost statistical significance following adjustment for insulin (HR_{q4-q1}, 1.48; 95% CI, 0.94–2.32; $P_{trend} = 0.09$).

Similarly, insulin was significantly associated with colorectal cancer, after adjustment for the established colorectal cancer risk factors (HR_{q4-q1}, 1.73; 95% CI, 1.16–2.57; $P_{trend} = 0.005$; Table 4), and additional adjustment for HT/estradiol and free IGF-I had no effect. Adjustment for waist circumference, however, weakened the association of insulin with colorectal cancer (HR_{q4-q1}, 1.42; 95% CI, 0.91–2.23; $P_{trend} = 0.11$), just as insulin attenuated the obesity-colorectal cancer relation. The HOMA-IR index had associations with colorectal cancer very similar to those of insulin itself (data not shown).

Free IGF-I levels were also associated with colorectal cancer in models that controlled for established risk factors, although the

Table 1. Selected baseline characteristics of the study population

Variable*	Cases (n = 438)	Subcohort† (n = 809)	Age-adjusted P value‡
Age, y (± SD)	65.93 (±7.2)	62.78 (±7.5)	<0.0001
Ethnicity, n (%)			
White	380 (87.2)	693 (86.0)	0.09
Black	35 (8.0)	52 (6.5)	
Hispanic	10 (2.3)	32 (4.0)	
Asian/other	11 (2.5)	29 (3.5)	
Weight, kg (±SD)	72.93 (±6.0)	71.40 (±6.7)	0.02
Body mass index, kg/m^2 (± SD)	27.56 (±5.3)	27.07 (±5.8)	0.045
Waist, cm (± SD)	87.47 (±14.5)	83.65 (±12.4)	<0.0001
Waist-to-hip ratio (± SD)	0.82 (±0.1)	0.80 (±0.1)	<0.0001
Currently using unopposed estrogen therapy, n (%)	88 (20.5)	186 (23.2)	0.56
Currently using combined estrogen + progestin therapy, n (%)	69 (16.1)	181 (22.6)	0.10
Currently using estrogen or estrogen + progestin transdermal patch, n (%)	3 (1.0)	12 (1.5)	0.89
NSAID use, n (%)	182 (41.6)	296 (36.6)	0.36
Family history of colorectal cancer, n (%)	80 (20.0)	126 (16.8)	0.25
History of colorectal polyps, n (%)	50 (23.9)	77 (17.6)	0.35
Smoking status, n (%)			
Never	193 (44.8)	432 (54.1)	0.002
Former	201 (46.6)	314 (39.3)	
Current	37 (8.6)	53 (6.6)	
Alcohol, servings per week, n (%)			0.50
0 ≤ 0.5	175 (40.1)	312 (38.6)	
0.5 ≤ 2.7	128 (29.3)	226 (28.0)	
≥2.7	134 (30.6)	270 (33.4)	
Physical activity, METs§ n (%)			0.01
<3.75	129 (29.7)	200 (24.9)	
3.75–9.82	109 (25.1)	196 (24.5)	
9.83–18.74	111 (25.6)	203 (25.3)	
≥18.75	85 (19.6)	203 (25.3)	
Serologic variables (± SD)‖			
Total IGF-I (ng/mL)	123.2 (±49.0)	119.8 (±48.4)	0.10
Free IGF-I (ng/mL)	0.33 (±0.36)	0.32 (±0.36)	0.94
IGFBP-3 (ng/mL)	4,114.2 (±812.8)	4,081.1 (±745.3)	0.75
Insulin (μIU/mL)	6.3 (±8.0)	5.2 (±4.7)	0.0001
Glucose (mg/dL)	92.0 (±10.2)	91.0 (±10.0)	0.46
Estradiol (pg/mL)	15.0 (±31.6)	17.0 (±35.1)	0.80

*Values are means (±SD) unless otherwise stated.
†For this cross-sectional analysis, the seven colorectal cancer cases that arose in the subcohort were excluded.
‡P values derived from age-adjusted Wilcoxon rank sum test for continuous data and Pearson's χ^2 for categorical data.
§MET, metabolic equivalent tasks (defined as the caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest) per hour per week.
‖Values are medians (±SD).

association was of borderline statistical significance (HR_{q4-q1}, 1.35; 95% CI, 0.92–1.98; P_{trend} = 0.05). This association was essentially unaltered following adjustment for waist circumference and HT/estradiol, but was attenuated following adjustment for insulin, (HR_{q4-q1}, 1.22; 95% CI, 0.82–1.81; P_{trend} = 0.17).

Unlike the above model, endogenous estradiol had a statistically significant positive association with risk of incident colorectal cancer that was entirely unaffected by controlling for waist, insulin, and free IGF-I, as well as all other colorectal risk factors. That is, the age-adjusted model (Table 3) and the full model that incorporated all other variables ($HR_{high/low}$, 1.53; 95% CI, 1.02–2.27), provided nearly identical estimates of the strength of association between endogenous estradiol levels and colorectal cancer. Use of estrogen and estrogen + progesterone, on the other hand, were both inversely, albeit nonsignificantly, associated with risk of incident colorectal cancer in age-adjusted models: the HR for use of estrogen compared with no hormone therapy was 0.83 (95% CI, 0.61–1.13) and was 0.77 (95% CI, 0.55–1.09) for use of estrogen + progesterone versus non–HT users (Table 3).

We detected no significant heterogeneity in the results stratified by HT use (estrogen or estrogen + progesterone), waist circumference, tumor location, including colon, versus rectum, or distal versus proximal colon, or tumor stage (all P-interaction > 0.5).

Discussion

In this large prospective investigation of postmenopausal women, we observed a statistically significant positive association of high endogenous estradiol levels with risk of incident colorectal cancer, after controlling for multiple other colorectal cancer risk factors. Three additional variables—waist circumference, insulin, and free IGF-I—were also significantly associated with risk of colorectal cancer in multivariate models, but these relationships each became attenuated and lost statistical significance when adjusted for one another. Together, these data suggest the existence of at least two independent biological pathways associated with the development of colorectal cancer: one that involves endogenous estradiol, and a second that is broadly associated with obesity, hyperinsulinemia, and free IGF-I.

The most novel of these findings was the positive association between endogenous estradiol and colorectal cancer. Although this result seems to conflict with the well established protective effects of exogenous hormone therapy (mainly estrogen + progesterone) against the development of colorectal cancer (23), we note that estrogen + progesterone use was associated, albeit nonsignificantly, with reduced risk of colorectal cancer in our data set. To our knowledge, no prior studies have assessed the relation of endogenous estradiol with incident colorectal cancer and, therefore, no other human data exist to either support or contradict this potentially important finding. Although this observation requires confirmation in other populations, the findings are biologically plausible. Several *in vitro* studies have shown that estrogen may have mitogenic and possibly tumorigenic effects on colorectal cells (34–42). For example, in human colorectal cancer cell lines, estradiol activates the mitogen-activated protein kinase cascade, a key pathway in the stimulation of DNA and protein synthesis that can induce cell growth and proliferation (36, 39). In addition, there is evidence of reduced metabolic inactivation of estradiol in colorectal cancer compared with normal tissue, suggesting that colon cancer cells may be exposed to higher levels of estradiol than noncancer cells (38, 42).

How then, if estradiol is tumorigenic, can we explain the protective effects of hormone therapy against the development of colorectal cancer? One possibility relates to the well known first-pass effect of oral estrogens on the liver, whereby oral hormone therapy exposes the liver to a large bolus of estrogen, altering hepatic protein synthesis (43). Indeed, our serologic data were consistent with prior reports of changes in the IGF-axis related to the first-pass effect: total IGF-I, free IGF-I, and IGFBP-3 levels were highest among women not using hormone therapy, lowest among women using estrogen, and intermediate among women using estrogen + progesterone (reflecting the fact that progestins partially offset the estrogen effects; refs. 43, 49). Insulin levels were also significantly lower in hormone therapy users in our data set, and a wide range of other factors may also be altered by the use of oral estrogen. For example, oral estrogen significantly increases C-reactive protein levels (50), decreases low-density lipoprotein, reduces lipoprotein(a) (50), and has a significant effect on coagulation-related proteins (51). The first-pass effect undoubtedly also causes a number of as yet undiscovered changes, and it remains unclear which factor(s) altered by the first-pass effect might be the most likely to explain the protective effects of oral estrogen + progesterone against colorectal cancer. Another possibility that has been raised relates to the fact that oral estrogen and estrogen + progesterone preparations are a concatenation of estrogens comprising mainly estrone rather than estradiol (42). Estrone has been shown to decrease the proliferative response in colonic epithelial cells, whereas estradiol promotes proliferation (36, 42), and estrone has been shown to protect ovariectomized mice from carcinogen-induced colon cancer (52). As a result, it has been hypothesized that the inverse relationship between hormone therapy and colorectal cancer could be partly attributed to the potentially protective effects of estrone. Direct measurement of other components of the sex hormone axis in addition to estradiol will be needed in future studies to more comprehensively address this issue.

The relationships of waist circumference (obesity), hyperinsulinemia, and free IGF-I with incident development of colorectal cancer were largely consistent with our a priori hypotheses; i.e., each of these three factors was positively associated with risk of colorectal cancer even after control for multiple other risk factors. However, when adjusted for one another, the effects of waist circumference, insulin, and free IGF-I each became attenuated and lost statistical significance. Specifically, we found that (*a*) control for insulin levels essentially eliminated the modest effects of free IGF-I, and moderately attenuated those of waist circumference;

Table 2. A Spearman's correlation matrix for age, waist circumference, and the serologic factors among the representative subcohort members

	Age	Waist	Total IGF-I	Free IGF-I	IGFBP-3	Insulin	Glucose	HOMA-IR
Age	—							
Waist	0.03	—						
Total IGF-I	−0.11	−0.02	—					
Free IGF-I	0.03	0.04	0.21*	—				
IGFBP-3	−0.04	0.10	0.44 †	0.14*	—			
Insulin	0.03	0.60*	0.07	0.11 †	0.16*	—		
Glucose	0.10	0.28	0.13*	0.11 †	0.12*	0.41*	—	
HOMA-IR	0.04	0.60*	0.08 ‡	0.12 †	0.17*	0.99*	0.52*	—
Estradiol§	−0.06	0.26*	0.04	−0.34*	−0.12	0.18*	0.07	0.17*

*$P < 0.001$.
†$P < 0.01$.
‡$P < 0.05$.
§Assessed in non-HT users only.

Table 3. Age-adjusted and multivariate associations (HRs and 95% CI) of baseline IGF-I, free IGF-I, IGFBP-3, insulin, glucose, HOMA-IR index, estradiol, hormone therapy use, and obesity with incident colorectal cancer

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* trend*
Total IGF-I					
Quartile cutpoints (ng/mL)	<94.2	94.2 to <119.6	119.6 to <151.0	≥151.0	
n (case/subcohort) †	113/203	104/205	113/205	108/203	
HR (95% CI) ‡	1.00§	0.90 (0.64–1.27)	1.09 (0.78–1.54)	1.04 (0.74–1.46)	0.58
Free IGF-I					
Quartile cutpoints (ng/mL)	<0.21	0.21 to <0.36	0.36 to <0.55	≥0.55	
n (case/subcohort) †	95/196	98/196	115/197	120/197	
HR (95% CI) ‡	1.00§	0.99 (0.69–1.42)	1.20 (0.85–1.70)	1.21 (0.86–1.72)	0.16
IGFBP-3					
Quartile cutpoints (ng/mL)	<3,604.0	3,604.0 to <4,053.6	4,053.6 to <4,583.5	≥4,583.5	
n (case/subcohort) †	125/203	79/204	114/203	112/205	
HR (95% CI) ‡	1.00§	0.65 (0.46–0.94)	1.00 (0.72–1.40)	0.98 (0.70–1.37)	0.62
Insulin					
Quartile cutpoints (μIU/mL)	<3.6	3.6 to <5.7	5.7 to <9.5	≥9.5	
n (case/subcohort) †	74/199	97/192	116/206	142/203	
HR (95% CI) ‡	1.00§	1.32 (0.90–1.91)	1.45 (1.00–2.09)	1.89 (1.33–2.69)	0.0005
Glucose					
Quartile cutpoints (mg/dL)	<86.0	86.0 to <91.5	91.5 to <100	≥100	
n (case/subcohort) †	99/200	108/210	98/195	129/207	
HR (95% CI) ‡	1.00§	0.94 (0.66–1.34)	0.91 (0.63–1.30)	1.16 (0.83–1.63)	0.40
HOMA-IR index					
Quartile cutpoints	<0.71	0.71 to <1.14	1.14 to <1.93	≥1.93	
n (case/subcohort) †	77/200	99/199	112/200	140/200	
HR (95% CI) ‡	1.00§	1.25 (0.86–1.81)	1.35 (0.94–1.94)	1.85 (1.30–2.64)	0.001
Estradiol‖					
Tertile cutpoints (pg/mL)	<8.0	8.0 to <14.0	≥14.0	N/A	
n (case/subcohort) †	68/149	122/158	83/135	N/A	
HR (95% CI) ‡	1.00§	1.67 (1.14–2.46)	1.43 (0.95–2.16)	N/A	0.09
Waist circumference					
Quartile cutpoints (cm)	<75.0	75.0 to <83.5	83.5 to <93.0	≥93.0	
n (case/subcohort) †	70/200	96/187	125/217	147/201	
HR (95% CI) ‡	1.00§	1.41 (0.97–2.08)	1.62 (1.13–2.34)	2.04 (1.43–2.92)	0.0001
Waist/hip ratio					
Quartile cutpoints	<0.75	0.75 to <0.80	0.80 to <0.85	≥0.85	
n (case/subcohort) †	83/200	87/200	122/202	145/201	
HR (95% CI) ‡	1.00§	1.00 (0.69–1.45)	1.29 (0.91–1.84)	1.47 (1.04–2.09)	0.01
BMI					
Category cutpoints¶ (kg/m^2)	<18.5	18.5 < 25.0	25.0 < 30.0	≥30	
n (case/subcohort) †	3/14	151/306	154/154	123/181	
HR (95% CI) ‡	0.32 (0.09–1.18)	1.00§	1.09 (0.81–1.45)	1.55 (1.13–2.13)	0.002
Unopposed estrogen	Nonuser	User			
n (case/subcohort) †	342/616	88/186	N/A	N/A	N/A
HR (95% CI) ‡	1.00§	0.83 (0.61–1.13)	N/A	N/A	N/A
Estrogen + progestin	Nonuser	User			
n (case/subcohort) †	361/621	69/181	N/A	N/A	N/A
HR (95% CI) ‡	1.00§	0.77 (0.55–1.09)	N/A	N/A	N/A

Abbreviation: N/A, not applicable.

*Significance tests for trend were calculated using ordinal quantile variables (1–4 for quartile and 1–3 for tertile) entered into the model as a single continuous variable.

†Total number of cases and subcohort vary slightly for each measured factor due to assay failure for some serum specimens.

‡Adjusted for age only.

§Referent category.

‖Among non-HT users only. Endogenous estradiol data (E2) were assessed only in women who did not use unopposed estrogen or combined (with progestin) hormone therapy because standard assays cannot accurately measure the equine hormones present in most hormone therapy (HT). This created five nonoverlapping groups, i.e., non-HT users with (*a*) low (referent), (*b*) moderate, or (*c*) high estradiol tertile levels, (*d*) estrogen users, (*e*) estrogen + progestin users. We then parameterized these groups as separate dummy variables with low estradiol as the common referent; permitting potentially different effects of estrogen use, estrogen + progestin use and high estradiol (among non–estrogen/estrogen + progestin users) to be modeled.

¶Parameterized according to the WHO's definition of overweight and obesity.

(*b*) control for waist circumference moderately attenuated the effects of insulin; but (*c*) adjustment for free IGF-I had little effect on either of the other two relationships. Together, these data suggest that confounding by insulin may account for the free IGF-I association.

Several possibilities may explain why including insulin and waist circumference in a single statistical model resulted in mutually attenuated but still moderate associations with colorectal cancer that were of borderline statistical significance for both variables. Most notably, obesity and hyperinsulinemia may share a tumorigenic pathway associated with risk of colorectal cancer, i.e., obesity may be associated with colorectal cancer, in part, because of the high prevalence of hyperinsulinemia in obese women and the direct tumorigenic effects of insulin on colorectal tissue. In this context, the degree to which adjustment for insulin alters the waist circumference hazard ratio could be interpreted as the extent to which the effects of obesity are accounted for by insulin, whereas the residual association of colorectal cancer with waist circumference is an indication that there are also additional obesity-related tumorigenic mechanisms. By extension, the existence of these additional pathways would also explain the attenuation of the insulin effect by adjustment for waist circumference, as obesity and insulin levels are correlated. We cannot, however, exclude the possibility that insulin and obesity are mainly biomarkers for unrecognized causal factors involved in colorectal tumorigenesis.

The possibility that insulin might have a direct etiologic role in the development of colorectal cancer is well supported by laboratory data. Insulin, in addition to its metabolic effects, has promitotic and antiapoptotic activity. For example, insulin binding to the IR leads to activation of IR substrate molecules and the downstream activation of the mitogen-activated protein kinase pathway. Furthermore, animal models have found that high insulin levels promote the development of aberrant crypt foci in the colon, which are posited to be colorectal cancer precursors (4), and overexpression of the IR can induce cell transformation *in vitro* (5).

Table 4. Multivariate associations (HRs and 95% CIs) of baseline waist circumference, insulin, free IGF-I, and endogenous estradiol with incident colorectal cancer

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* trend*
Waist circumference					
Multivariate model †	1.00 ‡	1.29 (0.85–1.95)	1.63 (1.08–2.45)	1.82 (1.22–2.70)	0.001
Multivariate + HT/E2	1.00 ‡	1.30 (0.85–1.99)	1.65 (1.09–2.51)	1.71 (1.12–2.59)	0.005
Multivariate + Insulin	1.00 ‡	1.17 (0.76–1.81)	1.42 (0.91–2.21)	1.48 (0.94–2.32)	0.09
Multivariate + free IGF-I	1.00 ‡	1.28 (0.84–1.95)	1.62 (1.07–2.47)	1.77 (1.18–2.64)	0.002
Full model§	1.00 ‡	1.15 (0.73–1.81)	1.45 (0.91–2.30)	1.37 (0.85–2.21)	0.15
Insulin					
Multivariate model †	1.00 ‡	1.23 (0.82–1.85)	1.43 (0.96–2.14)	1.73 (1.16–2.57)	0.005
Multivariate + HT/E2	1.00 ‡	1.26 (0.83–1.92)	1.44 (0.95–2.18)	1.72 (1.14–2.59)	0.007
Multivariate + waist	1.00 ‡	1.14 (0.74–1.75)	1.25 (0.81–1.92)	1.42 (0.91–2.23)	0.11
Multivariate + free IGF-I	1.00 ‡	1.22 (0.81–1.85)	1.42 (0.94–2.12)	1.69 (1.12–2.54)	0.008
Full model§	1.00 ‡	1.16 (0.74–1.81)	1.26 (0.80–1.98)	1.42 (0.89–2.28)	0.11
Free IGF-I					
Multivariate model †	1.00 ‡	1.08 (0.72–1.60)	1.41 (0.96–2.07)	1.35 (0.92–1.98)	0.05
Multivariate + HT/E2	1.00 ‡	1.06 (0.71–1.58)	1.37 (0.93–2.02)	1.28 (0.86–1.90)	0.09
Multivariate + waist	1.00 ‡	1.06 (0.71–1.59)	1.40 (0.95–2.06)	1.32 (0.89–1.95)	0.06
Multivariate + insulin	1.00 ‡	1.02 (0.68–1.52)	1.26 (0.85–1.87)	1.22 (0.82–1.81)	0.17
Full model§	1.00 ‡	1.00 (0.66–1.51)	1.25 (0.84–1.86)	1.18 (0.79–1.77)	0.21
Estradiol‖ ¶	Low	High			
Multivariate model †	1.00 ‡	1.53 (1.05–2.22)	N/A	N/A	N/A
Multivariate + waist	1.00 ‡	1.43 (0.97–2.11)	N/A	N/A	N/A
Multivariate + insulin	1.00 ‡	1.44 (0.98–2.12)	N/A	N/A	N/A
Multivariate + free IGF-I	1.00 ‡	1.64 (1.12–2.40)	N/A	N/A	N/A
Full model§	1.00 ‡	1.53 (1.02–2.27)	N/A	N/A	N/A

Abbreviation: N/A, not applicable.

*Significance tests for trend were calculated using ordinal quantile variables (1–4 for quartile and 1–3 for tertile) entered into the model as a single continuous variable.

†Multivariate model adjusted for age, smoking, ethnicity, family history of colorectal cancer, history of colonoscopy, physical activity, use of NSAIDs, and alcohol consumption.

‡Referent category.

§Multivariate model plus waist circumference, insulin, free IGF-I and HT/E2.

‖Among non-HT users only. Endogenous estradiol data (E2) were assessed only in women who did not use unopposed estrogen or combined (with progestin) hormone therapy because standard assays cannot accurately measure the equine hormones present in most hormone therapy (HT). This created five nonoverlapping groups, i.e., non-HT users with (*a*) low (referent), (*b*) moderate, or (*c*) high estradiol tertile levels, (*d*) estradiol users, (*e*) estrogen + progestin users. We then parameterized these groups as separate dummy variables with low estradiol as the common referent, permitting potentially different effects of estrogen use, estrogen + progestin use and high estradiol (among non–estrogen/estrogen + progestin users) to be modeled.

¶Parameterized as low (tertile 1) and high (combined tertiles 2 and 3).

Human colorectal adenocarcinomas have, in fact, been shown to express the IR at high levels, suggesting that these cells may be particularly sensitive to the tumorigenic effects of insulin (6).

A number of prior epidemiologic studies of colorectal cancer also suggested a possible association of hyperinsulinemia with risk of colorectal cancer. The preponderance of these positive studies reported a moderate 1.4- to 2.0-fold effect of hyperinsulinemia on colorectal cancer incidence, whether measured directly or through C-peptide. Although this effect was statistically significant more often in men (7, 10, 14, 16) than in women, most studies involved a relatively small number of female cases ($n < 200$; refs. 7–9, 13, 15), whereas a larger prospective investigation of women observed a near-significant 1.6-fold effect for colon cancer (17). The current study is one of the few to directly measure fasting insulin levels and also control for multiple colorectal cancer risk factors. Based on the cumulative data, it is fair to say that there is still growing but not yet conclusive evidence of a moderate effect of hyperinsulinemia on risk of colorectal cancer. However, there can be little remaining question regarding the existence of a tumorigenic pathway that is causally related to the risk of colorectal cancer and is at least correlated with obesity and hyperinsulinemia.

Our study has several important limitations that must be highlighted. Most notably, we measured only baseline serum levels, whereas repeated measurements over time would have provided more precise classification of subjects. It is possible, for example, that the attenuation of the baseline insulin effect, when adjusted for waist circumference, partly reflects the fact that both are actually surrogate markers of "long-term" insulin levels. In adults, though, the levels of insulin, total IGF-I, free IGF-I, IGFBP-3, and estradiol are known be stable over several years (53), suggesting that this issue would most likely have had a modest effect if any on the results during the 7-year follow-up in the current investigation. A second concern is that, despite having excluded women who developed colorectal cancer within the first 12 months of follow-up, we cannot entirely reject the possibility that some cases had subclinical disease at baseline, and that the associations we observed might be partly due to reverse causality. In this connection, we conducted a post hoc analysis that included only women who developed colorectal cancer at least 3 years after the baseline blood draw, and our results remained essentially unaltered (data not shown).

In summary, our data suggest the existence of at least two independent biological pathways associated with the development of colorectal cancer: one that involves endogenous estradiol, and a second that is broadly associated with obesity, hyperinsulinemia, and free IGF-I. If correct, strategies aimed at lowering endogenous estradiol levels, either through behavioral or pharmacologic means, could reduce the risk of colorectal cancer in postmenopausal women. Although a number of studies of the colorectal cancer-insulin relationship have been conducted without arriving at a firm conclusion, it may be of interest to now study individuals at known high risk of colorectal cancer, such as those with defined cancer-related gene mutations or a strong family history of colorectal cancer. The effects of insulin on colorectal tumorigenesis—which largely relate to cell proliferation and apoptosis—might well be strong in patients with DNA repair gene or other mutations.

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