Adipokines Linking Obesity with Colorectal Cancer Risk in Postmenopausal Women

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Abstract

Mechanistic associations between obesity and colorectal cancer remain unclear. In this study, we investigated whether adipokines are risk factors for colorectal cancer and whether they may mediate its association with obesity. In a case–cohort study nested within the Women's Health Initiative cohort of postmenopausal women, baseline plasma samples from 457 colorectal cancer cases and 841 subcohort subjects were assayed for seven adipokines—adiponectin, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin, hepatocyte growth factor, interleukin-6 (IL-6), and TNF-α. Serum insulin and estradiol values measured previously were also available for data analysis. After adjusting for age, race, smoking, colonoscopy history, and estrogen level, a low level of anti-inflammatory adiponectin and high levels of proinflammatory leptin, PAI-1, and IL-6 were associated with increased colorectal cancer risk, though only leptin remained significant after further adjustment for insulin [HRs comparing extreme quartiles ($HR_{Q4–Q1}$), 1.84; 95% CI, 1.17–2.90]. Mediation analyses showed that leptin and insulin partially explained the association between waist circumference and colorectal cancer and attenuated it by 25% and 37%, respectively, with insulin being a significant mediator ($P = 0.041$). Our findings support the conclusion that adipokines involved in inflammation are associated with colorectal cancer risk, but that their effects may be mediated mostly by insulin, with leptin exerting an independent effect. Hyperinsulinemia and hyperleptinemia may therefore partially explain the adiposity association with colorectal cancer in postmenopausal women. *Cancer Res; 72(12); 3029–37. ©2012 AACR.*

Introduction

Obesity, particularly central obesity, is a risk factor for colorectal cancer (1). A meta-analysis found a relative risk of 1.45 for the association between colorectal cancer and a relatively large waist circumference (2). Several mechanisms have been proposed to explain the obesity association with colorectal cancer (3, 4). First, obesity leads to insulin resistance and hyperinsulinemia, and insulin levels are positively associated with colorectal cancer risk (5–7). Second, adipose tissue secretes a variety of adipokines, and some of them are potent proinflammatory cytokines, such as interleukin (IL)-6 and TNF-α, which could promote tumor initiation and progression (8). Indeed, colorectal cancer is an inflammation-associated disease—elevated risk is seen among individuals with inflammatory bowel disease or a high level of the inflammation marker C-reactive protein (9, 10). Third, other adipokines produced by adipose tissue, such as leptin and adiponectin, have bioactivities affecting tumorigenesis, and some of these adipokines also play a critical role in regulating inflammation and insulin sensitivity (11). Although adipose tissue is the primary site for estrogen production after menopause (12), the role of endogenous estrogen in colorectal carcinogenesis and whether it provides a link between obesity with colorectal cancer risk are unclear. This is because postmenopausal hormone therapy use reduces the risk of colorectal cancer (13); normal colonic epithelial cells only express estrogen receptor β, which may have an antiproliferative effect (14); 2 prospective studies so far found inconsistent associations between estradiol and colorectal cancer risk (6, 15); more importantly, one of these prospective studies conducted in the same study population described in this report here showed that estradiol and obesity were independently associated with colorectal cancer, hence the obesity effects were not explained by endogenous estrogen (6).

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The list of WHI centers and investigators can be found online at http://www.whiscience.org/publications/WHI_investigators_shortlist_2005-2010.pdf.

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On the basis of the bioactivities of adipokines, it is hypothesized that they are associated with colorectal cancer risk and hence may provide a link between obesity and disease. Although several prospective studies have examined circulating levels of IL-6, TNF-α, leptin, and adiponectin in relation to colorectal cancer risk (16–23), none of them considered various components in the obesity pathway, namely hyperinsulinemia, inflammation, and adipokines, simultaneously, nor did they formally evaluate whether these components indeed mediate the effects of obesity.

We conducted a case–cohort study nested within the Women's Health Initiative Observational Study (WHI-OS). Our goals were (i) to assess the associations between colorectal cancer risk and 7 adipokines along with fasting insulin, which we previously measured in this study population and reported to have a significant positive association with colorectal cancer (6); and (ii) to examine whether these obesity-related analytes mediate the relationship between adiposity and colorectal cancer. The adipokines chosen were adiponectin, leptin, plasminogen activator inhibitor (PAI)-1, resistin, hepatocyte growth factor (HGF), TNF-α, and IL-6 because of their bioactivities and the feasibility of measuring them in multiplex assays with reasonable sensitivity and reliability (8, 24–27).

Patients and Methods

Study population

The WHI-OS is an ongoing prospective study with long-term follow-up of 93,676 postmenopausal women aged 50 to 79 years at recruitment, who were enrolled at 40 clinical centers in the United States from 1993 to 1998, to examine the risk factors for subsequent development of several health outcomes (28). At baseline, participants completed detailed epidemiologic questionnaires and a physical examination was conducted using standardized procedures to obtain various measurements, including height, weight, and waist circumference. Morning, fasting blood samples were collected, centrifuged, frozen onsite at −80°C, and later shipped to the central specimen repository. Diagnosis of colorectal cancer was ascertained by annual self-administered questionnaires and confirmed through centralized review of medical records.

Study subjects

This colorectal cancer study was part of a case–cohort study in which 3 cancer outcomes (breast, colorectum, and endometrium) were examined, and a representative subcohort served as the comparison group (6, 29, 30). By June 2004, 496 women had developed an incident primary tumor of the colon or rectum after the first year of follow up. The International Classification of Diseases for Oncology codes included 153.0–153.4, 153.6–153.9, and 154.0–154.1. The majority of the cases were adenocarcinomas (96%). The subcohort was created by randomly sampling 892 subjects from the WHI-OS participants who had more than 12 months of follow-up and had no history of breast, colorectal, or endometrial cancer at 12 months, regardless of their cancer outcome thereafter. Seven of the selected subcohort subjects developed incident colorectal cancer subsequently, and these 7 subjects were included in both the subcohort and case group. This unique feature of the case–cohort design was taken into consideration in data analyses described later. Ninety women taking diabetes treatment at baseline were excluded (39 cases and 51 subcohort) as these treatments may alter levels of the analytes under study. The final sample size included 457 colorectal cancer cases and 841 subcohort members (including 7 colorectal cancer cases). There were 373 cases of colon cancer, 82 cases of rectosigmoid junction or rectal cancer, and 2 cases with unknown location of the cancer.

Laboratory methods

EDTA plasma samples were assayed for 7 adipokines by the following methods: IL-6 by an ultrasensitive solid-phase ELISA [R&D Systems; interassay correlation of variation (CV) = 10%]; PAI-1, adiponectin, and resistin by a multiplex assay (Milliplex Human Adipokine Panel A; Millipore; interassay CVs of 12%, 13%, and 12%, respectively); leptin, HGF, and TNF-α by another multiplex assay (Milliplex Human Adipokine Panel B; Millipore; interassay CVs of 9%, 12%, and 13%, respectively). The interassay CVs were determined by including aliquots from 4 control samples in each of the 79 assay plates that were run over a 2-month period by the same technician; for each control sample, CV of a particular analyte was calculated as SD divided by the mean of analyte levels measured from the 79 aliquots. Average CVs of the 4 control samples are presented here. We previously measured and reported the results of fasting levels of serum insulin and estradiol in this study population (6), and data for these 2 analytes were included in multivariable data analyses reported here. The assay methods for insulin and estradiol were described previously (6). The intraclass correlation coefficients (ICC) of the 7 adipokines as well as those for insulin and estradiol were published previously. Based on 3 plasma samples collected over a 3-year period in 17 healthy women, we estimated the ICCs (shown in parentheses) to be: resistin (0.95), HGF (0.91), PAI-1 (0.84), adiponectin (0.73), insulin (0.62), leptin (0.58), IL-6 (0.47), and TNF-α (0.39; ref. 31), although a higher 3-year ICC for IL-6 (0.92) and TNF-α (0.88) had been reported in another study (32). Based on repeated samples from 79 postmenopausal women in the Nurses' Health Study, the 3-year ICC for estradiol was calculated to be 0.68 (33). These data suggest that a single measurement of circulating levels of the analytes under study in the baseline blood sample may reflect an individual's average levels over time.

Statistical analyses

In univariable analyses, we first examined the prevalences of established colorectal cancer risk factors (e.g., smoking, alcohol consumption, and inflammatory bowel disease, and so on) in cases and subcohort subjects. We then evaluated, among the subcohort subjects, whether various risk factors for colorectal cancer were associated with levels of the 7 adipokines using Wilcoxon rank-sum tests. In addition, correlations among the analytes and waist circumference were estimated by Spearman rank correlation coefficients in the subcohort. Throughout this report, we used waist circumference to indicate adiposity because it was a stronger risk factor for colorectal cancer than body mass index (BMI; ref. 6).

Multivariable analyses were conducted using Cox proportional hazard regression with robust variance estimation using the Self-Prentice method, which accounts for the case–cohort design in which cases may arise outside of or within the subcohort (34). We first developed a base model that retained only the baseline covariates age (continuous), race (Whites vs. others), and risk factors that were significantly associated with colorectal cancer in multivariable analyses in our study population—smoking status (never, former, or current), ever had a colonoscopy, and estrogen level in 4 categories [serum estradiol in tertiles among women who were not using hormone therapy (<8, 8–13.9, or ≥14 pg/mL) or women using hormone therapy at baseline]. Waist circumference and insulin level were excluded from the base model initially and analyzed together with the adipokines as described below. Colorectal cancer risk factors that were not statistically significant in multivariable modeling were excluded from the base model (e.g., family history of colorectal cancer, physical activity, and history of colorectal polyps, and so on). We first assessed the hazard ratios (HRs) for the association of each adipokine with colorectal cancer risk adjusting for the base model covariates. We then added waist circumference (continuous) and insulin level (continuous), which we previously reported to be linearly correlated with colorectal cancer risk (6), separately into the model to examine whether the adipokines had independent effects on colorectal cancer risk or their associations with colorectal cancer were partly explained by these 2 adiposity indicators. To ensure that these regression models with and without insulin and/or waist circumference had the same number of subjects and hence were comparable, we limited these analyses to individuals with complete data of the base model covariates, insulin, waist circumference, and the adipokine under analysis.

To account for the aggregate effect of multiple adipokines, an average adipokine score was computed by summing the quartile codings (0–3, in which 3 = highest quartile) of nonmissing adipokines and dividing by the number of adipokines. The coding for adiponectin was reversed (3 = lowest quartile) so that, similar to the other adipokines, the highest level was presumably associated with the greatest colorectal cancer risk. To avoid assuming any linear effect, all obesity-related analytes and the average adipokine score were categorized and analyzed in quartiles. Trend tests were carried out by analyzing the quartile categories as a continuous variable in the regression model.

In mediation analyses, we examined whether obesity-related analytes (adipokines and insulin) were mediators in the relationship between visceral adiposity, as indicated by waist circumference, and colorectal cancer. The hypothesized mediational process was as follows: waist circumference → obesity-related analyte → colorectal cancer. To qualify for a mediator, the obesity-related analyte needed to fulfill 3 conditions: (i) It was correlated with waist circumference. (ii) It was associated with colorectal cancer risk after adjusting for waist circumference. That is, the effects of the obesity-related analyte could not be explained by waist circumference because the analyte was presumably the one with the biological functions. (iii) On the contrary, the effects of waist circumference on colorectal cancer were partially or totally explained by the obesity-related analyte (see review in ref. 35). Criteria (i) and (ii) were assessed by Spearman rank correlation coefficients and Cox proportional hazard regression, respectively, as described above. For condition (iii), we quantified the extent of adiposity effects on cancer risk that could be explained by a potential mediator. We first entered waist circumference (continuous) into the base model and obtained the β coefficient for its association with colorectal cancer. An obesity-related analyte, which is a potential mediator, was then added into the model to obtain a β′ for waist circumference adjusting for that particular analyte. Percent attenuation of β due to a particular analyte was calculated as $100 \times (\beta\text{-}\beta')/\beta$. The method of Freedman and Schatzkin was used to test the null hypothesis that $\beta\text{-}\beta' = 0$ (35, 36). The caveat of the Freedman and Schatzkin method and all existing methods testing for mediation is that only one potential mediator can be statistically evaluated at a time.

All analyses were repeated after exclusion of cases with rectal cancer; the results were similar, and only data from the total study population are presented. *P* values shown are 2-sided.

Results

Table 1 shows the baseline prevalence of the established risk factors for colorectal cancer in the 457 cases and 834 subcohort subjects without colorectal cancer. As compared with the subcohort subjects, cases had higher BMI and waist circumference, and the between group difference in waist circumference was more pronounced than that in BMI. Cases were less physically active, older, and more likely to be smokers than the subcohort subjects; they were less likely to have had a colonoscopy, have ever used oral contraceptives, and have been a baseline hormone therapy user. Cases also had higher circulating levels of insulin and were more likely to have a medium level of estradiol than the subcohort subjects. In terms of the adipokines under study, cases had higher levels of leptin, PAI-1, HGF, and IL-6, but lower levels of adiponectin than the subcohort subjects (Table 1).

We also examined how the adipokines were associated with demographic factors (age and race) as well as colorectal cancer risk factors. Detailed data are included in a Supplementary Table. Briefly, adiposity was positively associated, whereas physical activity was negatively correlated, with levels of all adipokines except adiponectin, which showed associations in the opposite direction to those of the other analytes. Levels of most adipokines (adiponectin, resistin, HGF, TNF-α, and IL-6) increased with age. Hormone therapy was associated with decreased levels of leptin, PAI-1, resistin, HGF, TNF-α, and IL-6.

Table 2 shows that the analytes under study tended to be positively correlated with each other and with adiposity as indicated by waist circumference, except for adiponectin, which had negative correlations with the other factors. Most of the correlations were of moderate strength. The strongest correlations among the analytes were observed between insulin and leptin ($r = 0.58$) and between insulin and adiponectin ($r = -0.47$). Leptin ($r = 0.64$) and insulin ($r = 0.62$) had the

Table 1. Baseline characteristics in colorectal cancer cases and subcohort subjects without colorectal cancer from the WHI-OS cohort, 1994 to 2004

Baseline characteristics	Cases, *n* (%)	Subcohort, *n* (%)
Age, y		
50–54	33 (7.2)	137 (16.4)
55–56	146 (32.0)	354 (42.5)
65–74	215 (47.1)	291 (34.9)
75–79	63 (13.8)	52 (6.2)
Race ethnicity		
White	395 (86.8)	711 (85.7)
Black	38 (8.4)	57 (6.9)
Other	22 (4.8)	62 (7.5)
BMI (kg/m^2)		
15.5–24.9	155 (34.5)	322 (39.6)
25–29.9	158 (35.2)	296 (36.4)
30–68.8	136 (30.3)	196 (24.1)
Waist (cm)		
60.5–74.5	70 (15.3)	200 (24.1)
74.6–81.9	95 (20.8)	189 (22.8)
82.0–91.4	130 (28.5)	225 (27.1)
91.5–177.0	162 (35.5)	216 (26.0)
Physical activity (MET per week)		
0–3.7	134 (29.6)	206 (24.9)
3.8–10	113 (24.9)	208 (25.2)
10.1–19.9	117 (25.8)	207 (25.0)
20–113.2	89 (19.7)	206 (24.9)
Smoking status		
Never	203 (45.1)	442 (53.6)
Former	211 (46.9)	329 (39.9)
Current	36 (8.0)	53 (6.4)
Ever used oral contraceptives	133 (29.1)	340 (40.8)
Hormone therapy use at baseline		
No	295 (64.6)	454 (54.5)
Estrogen + progesterone	72 (15.8)	188 (22.6)
Estrogen alone	90 (19.7)	191 (22.9)
Lifetime duration of hormone therapy (years) among ever users		
<5	78 (33.2)	200 (38.8)
5–9	60 (25.5)	108 (21.0)
10–14	35 (14.9)	86 (16.7)
≥15	62 (26.4)	121 (23.5)
Family history of colorectal cancer		
No	334 (73.1)	645 (77.3)
Yes	83 (18.2)	129 (15.5)
Do not know	40 (8.8)	60 (7.2)
Ever had colonoscopy	220 (48.8)	464 (56.0)
Had polyps removed among those ever had colonoscopy	50 (23.3)	78 (17.3)

(Continued on the following column)

Table 1. Baseline characteristics in colorectal cancer cases and subcohort subjects without colorectal cancer from the WHI-OS cohort, 1994 to 2004 (Cont'd)

Baseline characteristics	Cases, *n* (%)	Subcohort, *n* (%)
Ever had inflammatory bowel disease	6 (1.3)	9 (1.1)
Used NSAID at least once a week	148 (32.4)	258 (30.9)
Alcohol servings per week		
0	187 (41.0)	327 (39.3)
0.1–1.56	132 (29.0)	233 (28.0)
1.57–66.3	137 (30.0)	273 (32.8)
Total folate intake (μg) per kcal per day		
0.09–0.29	128 (28.1)	202 (24.2)
0.30–0.40	111 (24.3)	206 (24.7)
0.41–0.58	104 (22.8)	211 (25.3)
0.59–3.26	113 (24.8)	215 (25.8)
Red meat intake (medium serving) per kcal per day × 10^3		
0–0.19	113 (24.8)	212 (25.4)
0.20–0.34	121 (26.5)	210 (25.2)
0.35–0.52	117 (25.7)	207 (24.8)
0.53–1.57	105 (23.0)	205 (24.6)
Serum estradiol level (pg/mL)[a]		
2.8–7.9	73 (25.4)	149 (33.4)
8–13.9	111 (38.7)	134 (30.0)
14–145.5	103 (35.9)	163 (36.6)
Levels of analytes	Median (IQR)	Median (IQR)
Insulin (uIU/mL)	6.5 (3.9–10.5)	5.3 (3.3–8.5)
Adiponectin (μg/mL)	27.2 (19.0–37.8)	29.1(20.4–39.8)
Leptin (ng/mL)	16.7 (8.7–30.0)	14.4 (7.0–24.7)
PAI-1 (ng/mL)	15.9 (10.8–23.8)	14.2 (9.0–20.8)
Resistin (ng/mL)	12.8 (9.9–16.4)	12.3 (9.8–15.6)
HGF (pg/mL)	653 (445–958)	597 (401–855)
TNF-α (pg/mL)	2.8 (1.9–3.9)	2.6 (1.8–3.6)
IL-6 (pg/mL)	1.6 (1.0–2.7)	1.4 (0.9–2.2)

Abbreviations: IQR, interquartile range; MET, metabolic equivalent of task; NSAID, nonsteroidal anti-inflammatory drugs.

[a]Among women not using hormone therapy at baseline.

highest correlation with waist circumference as compared with the other adipokines.

The multivariable associations of the 7 adipokines with colorectal cancer, after adjusting for age, race, smoking status, history of colonoscopy, and estrogen level in the base model, are shown in Table 3. Plasma levels of resistin, HGF, and TNF-α were not associated with colorectal cancer. Increased levels of adiponectin were inversely associated with colorectal cancer [HR comparing the highest versus lowest quartiles ($HR_{Q4–Q1}$), 0.65; 95% CI, 0.45–0.94)], whereas levels of leptin, PAI-1, and IL-6, were positively associated with colorectal cancer risk ($HR_{Q4–Q1}$ were 2.50, 1.87, and 1.41, respectively). After adjusting each

Table 2. Spearman rank correlations among obesity-related factors in subcohort subjects from the WHI-OS cohort, 1994 to 2004

	Leptin	PAI-1	Resistin	HGF	TNF-α	IL-6	Insulin	Estradiol[a]	Waist
Adiponectin	–0.30 (<0.001)	–0.21 (<0.001)	–0.004 (0.902)	–0.05 (0.159)	–0.03 (0.468)	–0.22 (<0.001)	–0.47 (<0.001)	–0.16 (0.001)	–0.35 (<0.001)
Leptin		0.35 (<0.001)	0.14 (<0.001)	0.17 (<0.001)	0.19 (<0.001)	0.34 (<0.001)	0.58 (<0.001)	0.22 (<0.001)	0.64 (<0.001)
PAI-1			0.08 (0.026)	0.24 (<0.001)	0.12 (<0.001)	0.26 (<0.001)	0.41 (<0.001)	0.07 (0.153)	0.39 (<0.001)
Resistin				0.05 (0.190)	0.20 (<0.001)	0.23 (<0.001)	0.16 (<0.001)	0.03 (0.585)	0.12 (<0.001)
HGF					0.34 (<0.001)	0.20 (<0.001)	0.16 (<0.001)	0.04 (0.345)	0.19 (<0.001)
TNF-α						0.23 (<0.001)	0.18 (<0.001)	–0.01 (0.883)	0.14 (<0.001)
IL-6							0.36 (<0.001)	0.15 (0.002)	0.45 (<0.001)
Insulin								0.18 (<0.001)	0.62 (0.001)
Estradiol									0.27 (<0.001)

NOTE: *P* values are shown in parentheses.
[a]Among women not using hormone therapy at baseline.

adipokine for either insulin level or waist circumference in the base model (Table 3), none of the analytes remained significantly associated with colorectal cancer risk, except for leptin ($HR_{Q4–Q1}$ adjusted for insulin, 1.84; 95% CI, 1.17–2.90; $HR_{Q4–Q1}$ adjusted for waist circumference, 1.76; 95% CI, 1.11–2.77). Although the tests for linear trend were significant for leptin, the risk for colorectal cancer did not rise with each increasing quartile of leptin but tended to dip in the third quartile.

To analyze all the analytes in the obesity pathway simultaneously, we entered leptin, the other 6 adipokines, and insulin into the base model (Table 4). For the ease of data presentation, an average adipokine score was calculated to represent an individual's aggregate exposure to the 6 nonleptin adipokines. The average adipokine score excluded leptin because our data so far indicated that the colorectal cancer association was distinct between leptin and the other 6 adipokines. By modeling them in 2 separate variables, we could examine whether the leptin effects were independent of all the other obesity-related analytes, and whether the other 6 adipokines aggregated together remained to have null effects. Table 4 shows that after accounting for the effects of other adipokines, leptin and insulin remained to be significant risk factors for colorectal cancer. Results were similar when the individual nonleptin adipokines categorized in quartiles, rather than the average adipokine score, were analyzed simultaneously; their associations with colorectal cancer remained null (data not shown).

To be a mediator in the relationship between visceral adiposity and colorectal cancer, an analyte needed to be (i) correlated with waist circumference, (ii) associated with colorectal cancer in the presence of waist circumference in the multivariable model and, at the same time, (iii) able to attenuate the effect of waist circumference. For criterion (i), we showed in Table 2 that leptin and insulin had the highest correlation with waist circumference. Only leptin and insulin fulfilled condition (ii): adjusting for the covariates in the base model and waist circumference, insulin was significantly associated with colorectal cancer risk ($HR_{continuous}$, 1.04; 95% CI, 1.01–1.08) and so was leptin (Table 3; $HR_{Q4–Q1}$, 1.76; 95% CI, 1.11–2.77). Leptin and insulin were formally evaluated in mediation analysis. The β coefficient for waist circumference adjusting for only the covariates in the base model was 0.0246 ($HR_{continuous}$, 1.02; 95% CI, 1.01–1.04). It was attenuated when the model was additionally adjusted for leptin (25% reduction in β, $P = 0.131$) or insulin (37% reduction, $P = 0.041$; Fig. 1). When leptin and insulin were entered into the model simultaneously, the β coefficient of waist circumference ($HR_{continuous}$, 1.01; 95% CI, 1.00–1.03) decreased 50%, but this 50% reduction in β could not be assessed for statistical significance because the statistical methods available so far could not be applied to multiple mediators.

Discussion

Adipokine dysregulation may account, in part, for the association between obesity and colorectal cancer risk (3, 4). Laboratory studies have shown that certain adipokines produced by adipose tissue have carcinogenic potential. Adipokines may promote tumor growth by acting as mitogens for normal and neoplastic colon cells (leptin, IL-6, and TNF-α) and by inhibiting apoptosis (PAI-1, leptin, IL-6, and TNF-α; refs. 8, 24, 25). HGF, PAI-1, leptin, IL-6, and TNF-α appear to have proangiogenic activity and promote neovascularization of tumors (8, 27, 37). HGF, PAI-1, IL-6, and TNF-α may be involved in tumor progression and metastasis through promotion of cell motility and invasion (8, 27, 37). In addition, adipokines may induce production of other adipokines and regulate inflammatory responses and insulin sensitivity (5–7, 9, 10). For examples, the proinflammatory cytokines IL-6 and TNF-α induce leptin and PAI-1 production (38); in turn, leptin and resistin upregulate secretion of proinflammatory cytokines (38, 39); and hyperleptinemia, IL-6, and TNF-α all contribute to hyperinsulinemia and insulin resistance (40, 41). Adiponectin, on the contrary, may exert beneficial effects by inhibiting cell growth and angiogenesis, suppressing secretion of inflammatory cytokines, and improving insulin sensitivity (42, 43).

In this study, we found that increased colorectal cancer risk was associated with relatively high circulating levels of adipokines that are involved in proinflammatory responses (leptin, IL-6, and PAI-1), whereas the anti-inflammatory adiponectin

Table 3. HRs for the associations of adipokines with colorectal cancer risk in the WHI-OS cohort, 1994 to 2004

	Q1	Q2	Q3	Q4	P_{trend}
Adiponectin (μg/mL)	<19.7	19.7–28.3	28.4–39.6	≥39.7	
# Cases/# subcohort	118/179	112/201	102/210	95/206	
Base model	1	0.85 (0.59–1.21)	0.72 (0.50–1.04)	0.65 (0.45–0.94)	0.015
Base model + insulin	1	1.01 (0.69–1.48)	0.96 (0.64–1.45)	0.94 (0.61–1.46)	0.740
Base model + waist circumference	1	0.93 (0.65–1.35)	0.88 (0.60–1.30)	0.86 (0.58–1.29)	0.448
Base model + insulin + waist circumference	1	1.01 (0.69–1.49)	1.00 (0.67–1.51)	1.02 (0.66–1.57)	0.952
Leptin (ng/mL)	<7.4	7.4–15.0	15.1–25.5	≥25.6	
# Cases/# subcohort	74/208	121/206	88/196	143/187	
Base model	1	1.73 (1.18–2.52)	1.42 (0.96–2.11)	2.50 (1.70–3.67)	<0.001
Base model + insulin	1	1.61 (1.10–2.36)	1.19 (0.78–1.81)	1.84 (1.17–2.90)	0.038
Base model + waist circumference	1	1.51 (1.02–2.23)	1.11 (0.72–1.72)	1.76 (1.11–2.77)	0.068
Base model + insulin + waist circumference	1	1.50 (1.02–2.21)	1.06 (0.69–1.63)	1.57 (0.98–2.51)	0.212
PAI-1 (ng/mL)	<9.2	9.2–14.4	14.5–21.3	≥21.4	
# Cases/# subcohort	67/198	114/199	110/195	122/181	
Base model	1	1.58 (1.07–2.32)	1.46 (0.98–2.16)	1.87 (1.27–2.76)	0.006
Base model + insulin	1	1.45 (0.98–2.15)	1.23 (0.82–1.86)	1.36 (0.87–2.11)	0.375
Base model + waist circumference	1	1.42 (0.96–2.11)	1.18 (0.78–1.80)	1.43 (0.93–2.19)	0.244
Base model + insulin + waist circumference	1	1.39 (0.94–2.07)	1.14 (0.75–1.73)	1.25 (0.80–1.96)	0.628
Resistin (ng/mL)	<10.0	10.0–12.5	12.6–15.7	≥15.8	
# Cases/# subcohort	104/204	96/204	106/194	121/195	
Base model	1	0.88 (0.61–1.27)	1.01 (0.70–1.45)	1.16 (0.81–1.65)	0.329
Base model + insulin	1	0.88 (0.61–1.27)	0.92 (0.63–1.33)	1.00 (0.69–1.46)	0.912
Base model + waist circumference	1	0.84 (0.57–1.22)	0.90 (0.62–1.32)	1.04 (0.72–1.50)	0.730
Base model + insulin + waist circumference	1	0.84 (0.58–1.23)	0.88 (0.60–1.29)	0.98 (0.68–1.43)	0.994
HGF (pg/mL)	<407.5	407.5–613.1	613.2–869.2	≥869.3	
# Cases/# subcohort	90/208	104/200	99/201	133/187	
Base model	1	1.09 (0.75–1.58)	1.09 (0.75–1.58)	1.26 (0.87–1.82)	0.232
Base model + insulin	1	1.12 (0.76–1.65)	1.07 (0.73–1.57)	1.15 (0.77–1.71)	0.556
Base model + waist circumference	1	1.11 (0.76–1.62)	1.02 (0.70–1.48)	1.11 (0.76–1.63)	0.709
Base model + insulin + waist circumference	1	1.12 (0.76–1.65)	1.03 (0.70–1.51)	1.09 (0.74–1.62)	0.776
TNF-α (pg/mL)	<1.9	1.9–2.6	2.7–3.6	≥3.7	
# Cases/# subcohort	93/193	100/200	100/191	119/189	
Base model	1	0.94 (0.65–1.36)	0.99 (0.69–1.44)	0.97 (0.66–1.42)	0.969
Base model + insulin	1	0.84 (0.57–1.24)	0.89 (0.61–1.30)	0.76 (0.50–1.16)	0.258
Base model + waist circumference	1	0.90 (0.62–1.32)	0.91 (0.62–1.33)	0.88 (0.60–1.29)	0.562
Base model + insulin + waist circumference	1	0.84 (0.57–1.24)	0.87 (0.59–1.27)	0.77 (0.51–1.17)	0.272
IL-6 (pg/mL)	<1.0	1.0–1.5	1.6–2.3	≥2.4	
# Cases/# subcohort	84/203	94/197	114/193	121/176	
Base model	1	0.98 (0.67–1.43)	1.17 (0.81–1.70)	1.41 (0.97–2.06)	0.043
Base model + insulin	1	0.86 (0.58–1.27)	0.98 (0.66–1.45)	1.04 (0.68–1.58)	0.662
Base model + waist circumference	1	0.80 (0.53–1.19)	0.89 (0.60–1.33)	0.91 (0.58–1.43)	0.914
Base model + insulin + waist circumference	1	0.78 (0.52–1.17)	0.87 (0.58–1.30)	0.84 (0.53–1.35)	0.690

NOTE: Base model adjusted for the following baseline covariates: age, race, smoking status, ever had colonoscopy, and estrogen level.

was inversely associated with risk. These observations are consistent with the idea that colorectal cancer is an inflammation-associated disease. However, the HRs for all of these adipokines were attenuated, and only leptin remained significant, after adjusting for insulin, suggesting that their effects on colorectal cancer risk are partly explained by insulin. Indeed, in mediation analyses examining insulin as a mediator in the relationships between these adipokines and colorectal cancer risk, we found that insulin significantly attenuated the β coefficient comparing the highest versus lowest quartiles ($\beta_{Q4\text{-}Q1}$) by 33% for leptin ($P = 0.018$), 51% for PAI-1 ($P < 0.001$), 86% for adiponectin ($P < 0.001$), and 90% for IL-6 ($P < 0.001$). Given that inflammation induces insulin resistance (40), and a high level of insulin is a risk factor for colorectal cancer

Table 4. Multivariable model for the associations of obesity-related analytes with colorectal cancer risk in the WHI-OS cohort, 1994 to 2004

	HR (95% CI)	*P*
Insulin (continuous, uIU/mL)	1.05 (1.01–1.09)	0.022
Leptin (ng/mL) in quartiles		
<7.4	1	0.024[a]
7.4–15.0	1.70 (1.15–2.51)	
15.1–25.5	1.26 (0.82–1.92)	
≥25.6	1.98 (1.24–3.15)	
Average adipokine score in quartiles[b]		
<1.1	1	0.474[a]
1.1–1.5	0.77 (0.53–1.12)	
1.6–1.8	0.77 (0.50–1.18)	
≥1.9	0.81 (0.50–1.30)	

NOTE: Multivariable model included base model covariates (age, race, smoking status, ever had colonoscopy, and estrogen level) + insulin + leptin + average adipokine score.

[a]P_{trend}.

[b]The average adipokine score was calculated based on 6 adipokines (adiponectin, PAI-1, resistin, HGF, IL-6, and TNF-α) excluding leptin (see Statistical Analyses section).

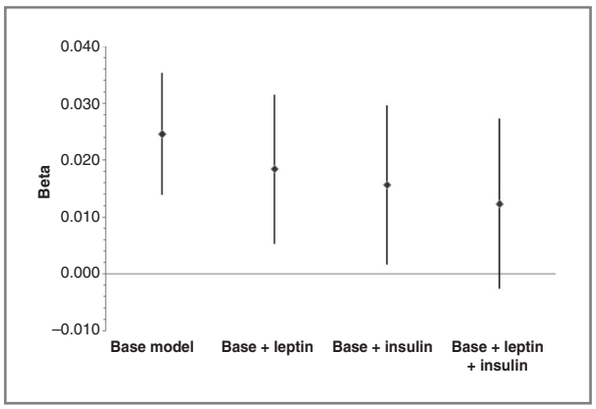

Figure 1. β coefficients for the association between waist circumference and colorectal cancer after accounting for possible mediation by leptin and/or insulin in the WHI-OS cohort, 1994 to 2004. Vertical lines represent the 95% CIs. Base model adjusted for baseline covariates (age, race, smoking status, ever had colonoscopy, and estrogen level).

(6, 7), it is reasonable to infer that hyperinsulinemia may mediate the relationship between inflammation and the development of colorectal cancer as illustrated in Fig. 2. Leptin was the only adipokine associated with colorectal cancer risk in this study after adjustment for insulin, proinflammatory cytokines (IL-6 and TNF-α), and the other adipokines (adiponectin, PAI-1, resistin, and HGF) under study. This suggests that hyperleptinemia may have independent effects on the development of colorectal cancer not simply explained by other inflammatory factors or insulin (Fig. 2). In addition, our data from mediation analyses suggest that hyperinsulinemia and hyperleptinemia may partly mediate the association between adiposity and colorectal cancer (Fig. 2). Nevertheless, other mediating factors in the obesity pathway remain to be identified.

Of the previous studies, only a handful of prospective studies have examined the effects of adipokines on colorectal cancer risk. The results for IL-6 in 3 studies and for TNF-α in one study were null (16, 17). Three prospective studies on circulating adiponectin levels had inconsistent results (18–20, 44). Three of 4 studies on leptin found a positive association with colorectal cancer, and 2 of these studies adjusted for both BMI and insulin (20–23). Unlike our data, none of these previous prospective studies considered simultaneously the effects of various components in the obesity pathway—adipokines, inflammation, and insulin. One cross-sectional study on colorectal adenoma reported a synergistic interaction between low levels of adiponectin and high levels of leptin after adjusting for BMI (45), but no interaction among the adipokines and insulin was observed in our study. Future studies are needed to confirm our findings. If leptin is confirmed to be an independent risk factor for colorectal cancer, intervention studies to reduce leptin levels may be warranted.

Our study has several limitations. Our results are not necessarily generalizable to men or to premenopausal women. In addition, inferences of the mediation analyses were partly based on cross-sectional data. The test for mediating effects

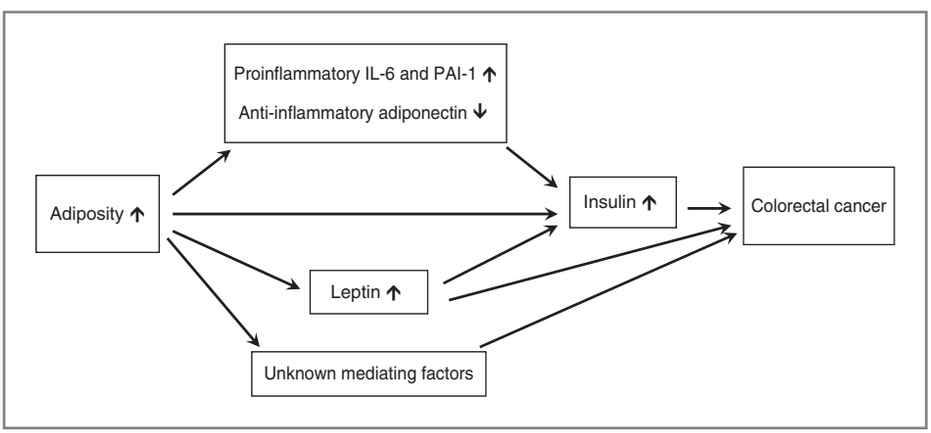

Figure 2. Hypothesis for biological mechanisms linking adiposity and colorectal cancer risk.

assumed a series of events from increased adiposity to subsequent high levels of adipokines and to eventual development of colorectal cancer. However, both adiposity and adipokines were measured cross-sectionally at baseline. As such, inferences of mediation analyses were based on the totality of our data and knowledge about the biological relationships among adiposity, adipokines, and carcinogenesis, while a temporal sequence of the first 2 events before cancer diagnosis was not established. Finally, the circulating levels of adipokines may not precisely reflect an individual's true exposure to adipokines. Such imprecision could be attributed to several factors. The multiplex assays for adipokines tend to have interassay CVs greater than 10%. Although the ICCs of the adipokines under study are reasonable for large-scale epidemiologic studies, using a single measurement of adipokine levels at baseline would inevitably be imprecise. Misclassification of adipokine levels due to these 2 factors, if nondifferential between cases and the subcohort, may have biased the results toward null. For adipokines that are also produced by mononuclear phagocytes (e.g., IL-6, TNF-α, and resistin), their levels in tissue as well as in the tumor microenvironment could be more relevant for tumor initiation and growth than their low circulating levels (8). The total levels of adiponectin, resistin, and PAI-1 were measured rather than their bioactive forms (27, 39, 46).

In summary, adipokines that are involved in inflammatory processes (a low level of adiponectin and high levels of IL-6, leptin, and PAI-1) are associated with risk of colorectal cancer, but their effects may be mediated through hyperinsulinemia. The exception is leptin, which appears to have its own independent effects. Hyperinsulinemia and hyperleptinemia may partially explain the association between obesity and colorectal cancer risk in postmenopausal women.

Disclosure of Potential Conflicts of Interest

S. Wassertheil-Smoller is a consultant and an advisory board member of Fred Hutchinson Cancer Research Center. No potential conflicts of interest were disclosed by the other authors.

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