

Interaction between Adiponectin and Leptin Influences the Risk of Colorectal Adenoma

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Abstract

Obesity has been associated with an increased risk of colorectal neoplasia, but the mechanisms of this potential association have not been elucidated. We hypothesized that the adipokines adiponectin, leptin, and tumor necrosis factor- α (TNF- α) may mediate an association between obesity and colorectal cancer. We measured plasma concentrations of total and high-molecular-weight (HMW) adiponectin, leptin, and TNF- α in healthy volunteer examinees who underwent total colonoscopy between February 2004 and February 2005, and conducted a case-control study consisting of 778 cases and 735 controls. An inverse association of total and HMW adiponectin was observed with colorectal adenoma (P trend < 0.001 and 0.03, respectively). Further, total adiponectin interacted with leptin, but not TNF- α , in relation to colorectal adenoma (P interaction = 0.007). An inverse association of total adiponectin with colorectal adenoma was apparent in the highest two tertiles of leptin, particularly the middle (P trend < 0.001), whereas a positive association of leptin was obvious in the lowest tertile of total adiponectin (P trend = 0.01) after adjusting for potential confounders and body mass index, which is a major determinant of insulin resistance. Adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, whereas leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. Our findings provide the first epidemiologic evidence for interactive effects of adiponectin and leptin in the early stage of colorectal tumorigenesis, distinct from their involvement in insulin resistance. *Cancer Res*; 70(13); 5430–7. ©2010 AACR.

Introduction

Overweight and obesity have been consistently associated with an increase in the risk of colorectal cancer and adenoma, a well-established precursor lesion of colorectal cancer (1). However, the mechanisms of this potential association between adiposity and colorectal neoplasia have not been fully elucidated. Adipose tissue, long considered an inert energy storage depot, is now recognized as an active endocrine organ, and in fact releases a wide variety of biologically functional molecules, collectively referred to as adipokines (2). Importantly, accumulating evidence suggests that several adipokines, namely adiponectin, leptin, and tumor necrosis factor- α (TNF- α), have the potential to mediate the association between adiposity and colorectal neoplasia (1). These adipokines are in fact all related to insulin resistance (2), which has been suggested to be an early and fundamental

disorder in the path to several obesity-related malignancies, including colorectal cancer (3).

Adiponectin, an insulin-sensitizing hormone, is secreted exclusively by adipocytes, and circulates in plasma in three forms of oligomeric complex: a simple complex of a trimer, a low-molecular-weight complex of two trimers, and a high-molecular-weight (HMW) complex of up to six trimers (3). Although HMW adiponectin is now considered the active form of the hormone, different forms have shown distinct biological effects through differential activation of downstream signaling cascades (3). Besides its well-known effect on insulin resistance, adiponectin seems to directly modulate several intracellular signaling pathways involved in colorectal carcinogenesis (4, 5), probably through the two isoforms of its receptors, adiponectin receptor 1 and 2, which are expressed in normal colon epithelium and colon cancer tissue (6, 7). Further, recent basic research has found that adiponectin inhibits leptin- and TNF- α -induced signaling cascades, both of which lead to cell proliferation and survival (8–11). However, few epidemiologic studies have examined the association of circulating levels of adiponectin with colorectal adenoma (12) and cancer (13–15), and no epidemiologic study has evaluated the interaction of adiponectin with leptin and TNF- α in relation to the risk of colorectal neoplasia.

Here, we measured plasma concentrations of total and HMW adiponectin, leptin, and TNF- α among middle-aged and elderly Japanese men and women, and investigated not only the association of circulating levels of these adipokines with colorectal adenoma but also the interaction of total and

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HMW adiponectin with leptin and TNF- α in relation to the risk of colorectal adenoma.

Materials and Methods

Study population

The Research Center for Cancer Prevention and Screening was established in 2004 as a branch of the National Cancer Center of Japan with the goal of developing preventive methods for various types of cancers. Among its efforts, the Research Center conducted the Colorectal Adenoma Study in Tokyo (16, 17), a case-control study specifically designed to investigate environmental and genetic factors related to the early stage of colorectal carcinogenesis among healthy volunteer examinees of a colorectal cancer screening. All examinees gave written informed consent to allow their data and materials collected through the screening to be used for medical research. The study protocol was approved by the institutional review board of the National Cancer Center.

Eligible subjects were defined in advance as men ages 50 to 79 years and women ages 40 to 79 years who underwent total colonoscopy from the anus to the cecum and who were without a history of colorectal adenoma, any malignant neoplasia, ulcerative colitis, Crohn's disease, familial adenomatous polyposis, carcinoid tumor, or colectomy. Of a consecutive series of 3,212 examinees undergoing magnifying colonoscopy with indigo carmine dye spraying between February 2004 and February 2005, 2,234 met these conditions. Based on the pit pattern of colorectal lesions, namely the characteristics of mucosal crypts, 526 men and 256 women were determined to have at least one adenoma and were thus included as adenoma cases. Pit-pattern classification based on magnifying chromo-endoscopy has been detailed elsewhere (18). Of the remaining 1,452 examinees, we identified 482 men and 721 women as potential controls who were also free from other benign lesions (e.g., hyperplastic polyps, inflammatory polyps, and diverticula). For efficiency, 256 of the potential female controls were frequency-matched to the female cases in five age categories (40–49, 50–54, 55–59, 60–64, and ≥ 65 years of age) and two screening periods (first and second halves). Because there were fewer potential male controls than male cases, all potential male controls were included in the study. Finally, the study enrolled 782 cases and 738 controls. Cases with adenomas of ≥ 5 mm in diameter were referred to clinical hospitals for definitive diagnosis and treatment.

Blood collection and laboratory procedures

Examinees were scheduled for blood collection before any cancer screening procedures on the first day of screening. Fasting venous blood was drawn into a vacutainer tube with EDTA. Almost three-quarters of examinees had fasted since the day before the screening day. The blood sample was centrifuged to obtain blood plasma and buffy coat, and these specimens were preserved at -80°C until analysis.

Plasma concentrations of total and HMW adiponectin were measured at Mitsubishi Chemical Medience, Tokyo, Japan, and those of leptin and TNF- α at GeneticLab, Hokkaido, Japan. All laboratory personnel were blinded with respect to

case and control status. Plasma concentrations of total and HMW adiponectin were simultaneously analyzed using a Human Adiponectin ELISA Kit for Total and Multimers (Sekisui Medical) by the enzyme-linked immunosorbent assay method. Minimum detection level was $0.39\ \mu\text{g}/\text{mL}$ for both total and HMW adiponectin. The kit manufacturer has reported that intra-assay coefficients of variation for total and HMW adiponectin are 5.4% and 5.0%, respectively. Plasma concentrations of leptin and TNF- α were simultaneously assayed using a Human Serum Adipokine (Panel B) LINCoplex Kit (Millipore) based on the xMAP Technology (Luminex). Minimum detection levels of leptin and TNF- α were 85.4 and $0.14\ \text{pg}/\text{mL}$, respectively. According to the manufacturer, the intra-assay coefficients of variation were reported to be 1.4% to 7.9%.

Self-administered questionnaire and anthropometric measurements

Before cancer screening, all examinees were encouraged to complete a self-administered questionnaire concerning lifestyle and socioeconomic characteristics as well as personal and family medical history. Details of the questionnaire have been described elsewhere (16, 17). In brief, the questionnaire inquired about smoking habits by first determining smoking status (current, past, and never) and then expressing lifetime exposure to cigarette smoking among ever smokers (i.e., past and current smokers) by pack-years, with 1 pack-year defined as the smoking of 20 cigarettes every day for 1 year. The questionnaire also inquired about drinking habits by first determining drinking status (current, past, and never) and then calculating the amount of alcohol consumed per week among current drinkers on the basis of the frequency of alcohol drinking and the number of standard units consumed per occasion for five different alcoholic beverages (sake, *shochu/awamori*, beer, whisky, and wine).

At the beginning of cancer screening, body weight and height were measured by medical personnel, and body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared.

Statistical analysis

An unconditional logistic regression model was used to estimate odds ratios (OR) and their 95% confidence intervals (95% CI) of colorectal adenoma according to sex-specific tertiles of total and HMW adiponectin, leptin, and TNF- α , with the lowest tertile for each adipokine used as the reference. Statistical adjustment was made in three models. Model 1 controlled for matching variables (i.e., age categories and screening periods) and the duration of fasting (from the day before the screening day, from the day of screening), whereas model 2 additionally adjusted for the following covariates: cigarette smoking (never, ≤ 20 , 21–40, and > 40 pack-years), alcohol drinking (never, past, < 150 , 150–299, ≥ 300 g/wk), family history of colorectal cancer (yes or no), and nonsteroidal anti-inflammatory drug use (yes or no). These covariates were suggested to be potential confounders in previous reports from the Colorectal Adenoma Study in Tokyo (16, 17). Model 3 further adjusted model 2 for BMI (< 21.0 , 21.0–22.9, 23.0–24.9, and $\geq 25.0\ \text{kg}/\text{m}^2$). Spearman's

Table 1. Selected characteristics of cases and controls by sex

Characteristic	Men			Women		
	Cases (n = 523)	Controls (n = 480)	P difference*	Cases (n = 255)	Controls (n = 255)	P difference*
Categorical variables, n (%)						
≥65 y of age	172 (33)	123 (26)	0.04	61 (24)	61 (24)	0.99
>40 pack-years	136 (26)	68 (14)	<0.001	6 (2)	2 (1)	0.03
≥300 g of alcohol/wk	153 (29)	98 (20)	0.004	6 (2)	8 (3)	0.14
Family history of CRC	72 (14)	65 (14)	0.91	55 (22)	26 (10)	<0.001
NSAID use	21 (4)	40 (8)	0.004	12 (5)	15 (6)	0.55
Overweight and obesity	188 (36)	124 (26)	0.002	46 (18)	37 (15)	0.31
Continuous variables, median (IQR)						
Total adiponectin (µg/mL)	3.98 (3.08–5.21)	4.37 (3.13–5.95)	0.002	6.81 (4.93–8.65)	7.36 (5.07–9.22)	0.21
HMW adiponectin (µg/mL)	1.20 (0.71–1.95)	1.33 (0.77–2.29)	0.02	2.78 (1.76–4.08)	3.01 (1.78–4.26)	0.28
Leptin (pg/mL)	3,333 (1,747–5,357)	2,671 (1,417–4,670)	<0.001	6,237 (3,789–10,739)	5,667 (3,138–9,260)	0.13
TNF-α (pg/mL)	2.70 (2.29–3.20)	2.67 (2.24–3.13)	0.42	2.45 (2.06–2.89)	2.50 (2.08–2.93)	0.42

Abbreviations: CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug; IQR, interquartile range.

*Based on the χ^2 test for percentage difference and the Wilcoxon rank-sum test for median difference.

correlation coefficients of BMI with total and HMW adiponectin, leptin, and TNF- α were -0.24, -0.23, 0.59, and 0.06, respectively, for male controls, and -0.21, -0.22, 0.64, and 0.18, respectively, for female controls. Linear trends in the ORs of colorectal adenoma were also assessed by assigning ordinal values to tertiles of respective adipokines. Finally, we combined men and women according to sex-specific tertiles of total and HMW adiponectin, leptin, and TNF- α , and examined whether the association between these adipokines and colorectal adenoma was modified by sex. Interaction terms were created between indicator variables representing categories of each adipokine and of sex, and their significance was statistically evaluated based on the likelihood ratio test with two degrees of freedom.

We then examined whether adiponectin interacted with leptin or TNF- α to modify its association with colorectal adenoma. We obtained ORs and 95% CIs of colorectal adenoma for nine combinations of tertiles of adiponectin and of leptin/TNF- α , with reference to the combination of the lowest tertile of adiponectin and the highest tertile of leptin/TNF- α . Finally, we statistically evaluated these interactions based on the likelihood ratio test with four degrees of freedom. Interaction terms were created between indicator variables representing tertiles of adiponectin and of leptin/TNF- α .

Of 1,520 study subjects, 7 had missing information, namely 3 with regard to cigarette smoking and 4 for BMI. These were then excluded, and the current analysis was conducted in 1,003 men (523 cases, 480 controls) and 510 women (255 cases, 255 controls). Of these, 121 and 57 had plasma concentrations of HMW adiponectin and leptin below the minimum detection levels, respectively, and were assigned the putative

values of 0.30 µg/mL and 50.0 pg/mL, respectively. Two-sided *P* values <0.05 were regarded as statistically significant. All statistical analyses were carried out using Statistical Analysis System (SAS), version 9.1 (SAS Institute).

Results

Selected characteristics of cases and controls by sex

Table 1 summarizes selected characteristics of cases and controls by sex. Male cases were more likely to be old and overweight, and tended to consume more cigarettes and alcohol, whereas male controls tended to use more nonsteroidal anti-inflammatory drugs. Female controls were more likely to be never smokers and tended to have less family history of colorectal cancer than female cases. Table 1 also shows plasma concentrations of total and HMW adiponectin, leptin, and TNF- α among cases and controls by sex. Male cases had lower plasma concentrations of total and HMW adiponectin and higher plasma concentrations of leptin than male controls. Of note, we observed substantial sex difference in plasma concentrations of total and HMW adiponectin and leptin. Correlations between total and HMW adiponectin, leptin, and TNF- α are presented in Supplementary Table S1. Total and HMW adiponectin were weakly inversely correlated with leptin, whereas leptin was weakly positively correlated with TNF- α .

Association of total and HMW adiponectin with colorectal adenoma

Table 2 shows the ORs of colorectal adenoma according to sex-specific tertiles of total and HMW adiponectin. In men, we observed a statistically significant trend of decreasing

adjusted ORs for colorectal adenoma across tertiles of total adiponectin (P trend = 0.002), and a marginally significant trend for HMW adiponectin (P trend = 0.08). A significantly reduced OR was also seen among men in the highest tertile of total adiponectin. Adjusted ORs of colorectal adenoma for the highest compared with the lowest tertile were 0.60 (95% CI, 0.44–0.83) and 0.75 (95% CI, 0.54–1.03) for total and HMW adiponectin, respectively. On further adjustment for BMI, the inverse association between total adiponectin and colorectal adenoma was still evident (P trend = 0.01). In women, neither total nor HMW adiponectin was measurably associated with colorectal adenoma, although adjusted ORs of colorectal adenoma for the highest tertile were below unity for both forms of adiponectin. When men and women were combined according to sex-specific tertiles, a significant trend of decreasing adjusted ORs across tertiles was observed for both total and HMW adiponectin (P trend < 0.001 and 0.03,

respectively). Although additional adjustment for BMI attenuated the inverse association between both forms of adiponectin and colorectal adenoma, a significant trend across tertiles remained for total adiponectin (P trend = 0.01). The inverse association of total adiponectin remained significant after further adjustment for indicators of energy balance (i.e., total energy intake, physical activity, and height), dietary factors (i.e., intakes of meat; fruits and vegetables; dairy products; folate; vitamins B₂, B₆, and B₁₂; vitamin D; calcium; and total isoflavones), and metabolic factors (i.e., serum concentrations of triglycerides, total cholesterol, and glucose; P trend = 0.02; data not shown). When total and HMW adiponectin levels were treated as a continuous variable in model 2, adjusted ORs of colorectal adenoma for a 1 μ g/mL increase were 0.95 (95% CI, 0.92–0.99) and 0.94 (95% CI, 0.88–1.01) for total and HMW adiponectin, respectively (data not shown). In this analysis of HMW adiponectin, 121 subjects

Table 2. Association of total and HMW adiponectin with colorectal adenoma

Measurement	Tertile			<i>P</i> trend*
	Lowest OR (95% CI)	Middle OR (95% CI)	Highest OR (95% CI)	
Total adiponectin				
Men, range (μ g/mL)	–3.64	3.65–5.26	5.27–	
Model 1 [†]	1.00 (reference)	0.79 (0.59–1.07)	0.55 (0.40–0.76)	<0.001
Model 2 [‡]	1.00 (reference)	0.83 (0.61–1.13)	0.60 (0.44–0.83)	0.002
Model 3 [§]	1.00 (reference)	0.85 (0.62–1.15)	0.66 (0.47–0.92)	0.01
Women, range (μ g/mL)	–5.76	5.77–8.49	8.50–	
Model 1 [†]	1.00 (reference)	1.01 (0.66–1.53)	0.69 (0.44–1.08)	0.11
Model 2 [‡]	1.00 (reference)	1.05 (0.68–1.61)	0.80 (0.50–1.27)	0.36
Model 3 [§]	1.00 (reference)	1.07 (0.69–1.65)	0.88 (0.54–1.41)	0.61
Men and women combined				0.68
Model 1 [†] [¶]	1.00 (reference)	0.86 (0.67–1.09)	0.60 (0.46–0.77)	<0.001
Model 2 [‡] [¶]	1.00 (reference)	0.87 (0.68–1.11)	0.64 (0.49–0.83)	<0.001
Model 3 [§] [¶]	1.00 (reference)	0.89 (0.69–1.14)	0.70 (0.53–0.91)	0.01
HMW adiponectin				
Men, range (μ g/mL)	–0.88	0.89–1.91	1.92–	
Model 1 [†]	1.00 (reference)	1.04 (0.77–1.41)	0.71 (0.52–0.98)	0.04
Model 2 [‡]	1.00 (reference)	1.05 (0.78–1.43)	0.75 (0.54–1.03)	0.08
Model 3 [§]	1.00 (reference)	1.08 (0.79–1.47)	0.82 (0.59–1.15)	0.28
Women, range (μ g/mL)	–2.19	2.20–3.90	3.91–	
Model 1 [†]	1.00 (reference)	1.13 (0.74–1.71)	0.75 (0.48–1.18)	0.22
Model 2 [‡]	1.00 (reference)	1.17 (0.76–1.80)	0.85 (0.54–1.36)	0.52
Model 3 [§]	1.00 (reference)	1.20 (0.78–1.87)	0.94 (0.58–1.53)	0.85
Men and women combined				0.93
Model 1 [†] [¶]	1.00 (reference)	1.07 (0.84–1.36)	0.73 (0.56–0.94)	0.01
Model 2 [‡] [¶]	1.00 (reference)	1.07 (0.83–1.36)	0.75 (0.58–0.97)	0.03
Model 3 [§] [¶]	1.00 (reference)	1.10 (0.85–1.40)	0.83 (0.63–1.08)	0.19

*Statistical tests for trend (two-sided) were assessed by assigning ordinal values to tertiles of each measurement.

[†]Adjusted for age, screening period, and duration of fasting.

[‡]Model 1 + cigarette smoking, alcohol drinking, family history of colorectal cancer, and nonsteroidal anti-inflammatory drug use.

[§]Model 2 + BMI.

^{||}Values are P interaction instead of P trend.

[¶]Further adjusted for sex.

below the minimum detection levels were excluded. Despite the sex differences in plasma concentrations of adiponectin, a significant effect modification by sex was not seen for either total or HMW adiponectin (P interaction = 0.68 and 0.93, respectively).

Association of leptin and TNF- α with colorectal adenoma

We also investigated the association of leptin and TNF- α with colorectal adenoma (Table 3). When men and women were combined according to sex-specific tertiles of leptin, a significant trend of increasing adjusted ORs across tertiles was observed (P trend < 0.001) with a significantly elevated OR for the highest tertile (OR, 1.57; 95% CI, 1.21–2.02). On additional adjustment for BMI, the positive association between leptin and colorectal adenoma was considerably

attenuated (P trend = 0.10). In contrast, no material association was seen between TNF- α and colorectal adenoma. When leptin and TNF- α levels were treated as a continuous variable in model 2, adjusted ORs of colorectal adenoma for a 1 ng/mL increase in leptin and a 1 pg/mL increase in TNF- α were 1.03 (95% CI, 1.01–1.05) and 0.99 (95% CI, 0.96–1.02), respectively (data not shown). In this analysis of leptin, 57 subjects below the minimum detection levels were excluded. Again, effect modification by sex was not observed for either leptin or TNF- α (P interaction = 0.53 and 0.42, respectively).

Association of total and HMW adiponectin with colorectal adenoma according to tertiles of leptin and TNF- α

We then examined whether adiponectin interacted with leptin or TNF- α to modify its association with colorectal

Table 3. Association of leptin and TNF- α with colorectal adenoma

Measurement	Tertile			P trend*
	Lowest OR (95% CI)	Middle OR (95% CI)	Highest OR (95% CI)	
Leptin				
Men, range (pg/mL)	–1,756	1,757–3,842	3,843–	
Model 1 [†]	1.00 (reference)	1.29 (0.94–1.78)	1.69 (1.24–2.30)	0.001
Model 2 [‡]	1.00 (reference)	1.30 (0.94–1.80)	1.73 (1.26–2.38)	<0.001
Model 3 [§]	1.00 (reference)	1.18 (0.84–1.67)	1.44 (0.99–2.08)	0.05
Women, range (pg/mL)	–3,856	3,857–7,908	7,909–	
Model 1 [†]	1.00 (reference)	1.31 (0.85–2.03)	1.36 (0.88–2.10)	0.18
Model 2 [‡]	1.00 (reference)	1.23 (0.78–1.93)	1.36 (0.87–2.13)	0.18
Model 3 [§]	1.00 (reference)	1.15 (0.71–1.86)	1.11 (0.65–1.92)	0.70
Men and women combined				0.53
Model 1 [†] [¶]	1.00 (reference)	1.30 (1.00–1.67)	1.55 (1.21–2.00)	<0.001
Model 2 [‡] [¶]	1.00 (reference)	1.28 (0.99–1.66)	1.57 (1.21–2.02)	<0.001
Model 3 [§] [¶]	1.00 (reference)	1.17 (0.89–1.54)	1.29 (0.95–1.74)	0.10
TNF-α				
Men, range (pg/mL)	–2.38	2.39–2.97	2.98–	
Model 1 [†]	1.00 (reference)	1.19 (0.87–1.62)	1.01 (0.74–1.38)	0.97
Model 2 [‡]	1.00 (reference)	1.24 (0.90–1.69)	0.97 (0.70–1.34)	0.85
Model 3 [§]	1.00 (reference)	1.24 (0.90–1.70)	0.94 (0.68–1.30)	0.70
Women, range (pg/mL)	–2.22	2.23–2.79	2.80–	
Model 1 [†]	1.00 (reference)	0.98 (0.64–1.49)	0.74 (0.47–1.15)	0.18
Model 2 [‡]	1.00 (reference)	0.88 (0.56–1.37)	0.69 (0.43–1.10)	0.11
Model 3 [§]	1.00 (reference)	0.85 (0.54–1.33)	0.65 (0.41–1.05)	0.07
Men and women combined				0.42
Model 1 [†] [¶]	1.00 (reference)	1.11 (0.87–1.42)	0.91 (0.71–1.18)	0.47
Model 2 [‡] [¶]	1.00 (reference)	1.15 (0.89–1.48)	0.88 (0.68–1.14)	0.34
Model 3 [§] [¶]	1.00 (reference)	1.13 (0.88–1.46)	0.85 (0.65–1.10)	0.21

*Statistical tests for trend (two-sided) were assessed by assigning ordinal values to tertiles of each measurement.

[†]Adjusted for age, screening period, and duration of fasting.

[‡]Model 1 + cigarette smoking, alcohol drinking, family history of colorectal cancer, and nonsteroidal anti-inflammatory drug use.

[§]Model 2 + BMI.

^{||}Values are P interaction instead of P trend.

[¶]Further adjusted for sex.

Table 4. Association of total adiponectin with colorectal adenoma according to tertiles of leptin and TNF- α

Measurement	Tertiles for total adiponectin*			<i>P</i> trend [†]
	Lowest OR (95% CI)	Middle OR (95% CI)	Highest OR (95% CI)	
Leptin [‡] §				0.007
Highest tertile	1.00 (reference)	0.78 (0.52–1.15)	0.70 (0.44–1.09)	0.05
Middle tertile	1.02 (0.68–1.53)	0.85 (0.57–1.28)	0.40 (0.25–0.64)	<0.001
Lowest tertile	0.52 (0.32–0.84)	0.69 (0.43–1.09)	0.71 (0.45–1.10)	0.21
TNF- α [§] ¶				0.20
Highest tertile	1.00 (reference)	1.03 (0.68–1.58)	0.57 (0.35–0.93)	0.04
Middle tertile	1.33 (0.87–2.02)	1.00 (0.65–1.55)	1.19 (0.76–1.86)	0.96
Lowest tertile	1.24 (0.80–1.93)	1.14 (0.74–1.76)	0.77 (0.49–1.21)	0.01

*Cutoff points were 3.64 and 5.26 $\mu\text{g/mL}$ for men and 5.76 and 8.49 $\mu\text{g/mL}$ for women.

[†]Statistical tests for trend (two-sided) were assessed by assigning ordinal values to tertiles of each measurement.

[‡]Cutoff points were 1,756 and 3,842 pg/mL for men and 3,856 and 7,908 pg/mL for women.

[§]Adjusted for age, screening period, duration of fasting, sex, cigarette smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, and BMI.

^{||}Values are *P* interaction instead of *P* trend.

[¶]Cutoff points were 2.38 and 2.97 pg/mL for men and 2.22 and 2.79 pg/mL for women.

adenoma. In this analysis, men and women were combined according to sex-specific tertiles of adiponectin, and stratified by leptin and TNF- α , respectively, based on sex-specific tertiles for controls. We observed a statistically significant interaction of total adiponectin with leptin (*P* interaction = 0.007), but not with TNF- α (*P* interaction = 0.20; Table 4). Compared with those in the lowest tertile of total adiponectin and highest tertile of leptin, those in the lowest tertiles of total adiponectin and leptin showed a statistically significant decrease in OR for colorectal adenoma (OR, 0.52; 95% CI, 0.32–0.84). However, a further decrease in ORs was not seen with increasing levels of total adiponectin among those in the lowest tertile of leptin (*P* trend = 0.21). In contrast, those in the middle and highest tertiles of leptin showed an inverse association between total adiponectin and colorectal adenoma. An inverse association was more prominent among those in the middle tertile of leptin (*P* trend < 0.001), with a significantly reduced OR of colorectal adenoma for the highest tertile of total adiponectin (OR, 0.40; 95% CI, 0.25–0.64). Of note, increasing levels of leptin were associated with elevated ORs of colorectal adenoma only among those in the lowest tertile of total adiponectin (*P* trend = 0.01; data not shown). After adjustment for BMI and other potential confounders, ORs for the lowest, middle, and highest tertiles of leptin were 1.00 (reference), 1.96 (95% CI, 1.21–3.17), and 1.92 (95% CI, 1.19–3.11), respectively, in the lowest tertile of total adiponectin (data not shown). If the above analysis of total adiponectin and leptin was repeated without interaction terms, mutually adjusted ORs of colorectal adenoma for the lowest, middle, and highest tertiles were 1.00 (reference), 0.90 (95% CI, 0.70–1.15), and 0.71 (95% CI, 0.54–0.93), respectively, for total adiponectin, whereas the corresponding va-

lues were 1.00 (reference), 1.13 (95% CI, 0.86–1.49), and 1.25 (95% CI, 0.92–1.69), respectively, for leptin (data not shown). In accordance with the above results, we observed a marginally significant interaction of HMW adiponectin with leptin (*P* interaction = 0.07), but not with TNF- α (*P* interaction = 0.21; Table 5). Again, these results were not essentially changed by additional adjustment for indicators of energy balance, dietary factors, and metabolic factors (*P* interaction with leptin = 0.006 and 0.07 for total and HMW adiponectin, respectively; data not shown). Results were essentially the same when the above analysis was conducted for men and women separately (*P* interaction of total adiponectin with leptin = 0.04 and 0.01 for men and women, respectively; data not shown).

Discussion

In this study, we observed an inverse association between total adiponectin and colorectal adenoma with statistical significance. This association remained significant, albeit considerably attenuated, after further adjustment for BMI, a major determinant of insulin resistance (2), suggesting that adiponectin may decrease the risk of colorectal neoplasia through mechanisms other than the indirect mechanism through insulin resistance. We also observed an inverse association of HMW adiponectin with colorectal adenoma, although significance was lost with additional adjustment for BMI. HMW adiponectin has a potent insulin-sensitizing effect, whereas circulating levels of HMW adiponectin and the degree of insulin sensitivity are determined mainly by the amount of adipose tissue (2, 3). Given that improved insulin sensitivity has been related to a decreased risk of

colorectal neoplasia (1), the HMW form of adiponectin may mediate the association between adiposity and colorectal neoplasia through its well-recognized influence on insulin resistance.

Our observations for colorectal adenoma agree with those for colorectal cancer from a case-control study nested in the Health Professionals Follow-up Study (13), in which a statistically significant inverse association was seen between plasma adiponectin level and the risk of colorectal cancer. Several clinical studies have also provided supportive evidence that patients with colorectal neoplasia had lower circulating levels of adiponectin than controls, although these studies were small (19–21). However, circulating adiponectin levels were not associated with risk in a case-control study of colorectal adenoma in a Japanese population (12) or in nested case-control studies of colorectal cancer in Norwegian and Swedish populations (14, 15). In contrast, the only epidemiologic investigation of HMW adiponectin in relation to the risk of colorectal neoplasia reported results inconsistent with ours (12). To date, epidemiologic evidence for the association of total and HMW adiponectin with colorectal neoplasm is both sparse and controversial, and further studies to corroborate our results are needed.

To our knowledge, this is the first study to provide epidemiologic evidence that adiponectin and leptin interact to modify the risk of colorectal adenoma separate to their profound involvement in insulin resistance. After adjusting for BMI and other potential confounders, an inverse association of adiponectin with colorectal adenoma was apparent in the highest two tertiles of leptin, particularly the middle, whereas a positive association of leptin was obvious in the lowest ter-

tile of adiponectin. A recent basic research study in a model of preneoplastic colon epithelial cells analogously showed that adiponectin inhibited multiple signaling cascades associated with leptin-induced cell proliferation (8). These findings lead to the hypotheses that adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, and that leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. This interaction would be independent to their well-documented influences on insulin resistance. These hypotheses require further interdisciplinary examination.

Among the strengths of the present study, the provision of total colonoscopy to all study subjects likely decreased the possibility of misclassification between cases and controls. Also, the number of subjects was considerably larger than in previous studies of the association between circulating levels of adiponectin and colorectal neoplasia (12–15).

A major limitation of this study is its cross-sectional nature, and the observed associations might be due to reverse causality. In contrast to colorectal cancer, however, it is unlikely that colorectal adenoma affects the amount of adipose tissue, a major determinant of circulating adiponectin levels (3), because colorectal adenoma is an asymptomatic benign tumor. A second limitation is the relatively small body size of the study population: Given that median BMI for male and female controls was 23.4 and 21.8 kg/m², respectively, and the prevalence of overweight and obesity was 26% and 15%, respectively, our observations may not be directly applicable to severely obese populations, often found in North American and European countries, where more than half of adults are overweight or obese (22).

Table 5. Association of HMW adiponectin with colorectal adenoma according to tertiles of leptin and TNF- α

Measurement	Tertiles for HMW adiponectin*			P trend [†]
	Lowest OR (95% CI)	Middle OR (95% CI)	Highest OR (95% CI)	
Leptin [‡] §				0.07
Highest tertile	1.00 (reference)	1.03 (0.70–1.53)	0.73 (0.47–1.15)	0.16
Middle tertile	1.00 (0.66–1.51)	0.93 (0.62–1.39)	0.60 (0.38–0.94)	0.05
Lowest tertile	0.49 (0.30–0.82)	0.87 (0.55–1.37)	0.77 (0.50–1.20)	0.13
TNF- α [§] ¶				0.21
Highest tertile	1.00 (reference)	1.47 (0.96–2.26)	0.76 (0.47–1.22)	0.36
Middle tertile	1.46 (0.95–2.23)	1.39 (0.90–2.13)	1.46 (0.93–2.31)	0.62
Lowest tertile	1.45 (0.92–2.29)	1.37 (0.89–2.11)	1.03 (0.65–1.61)	0.06

*Cutoff points were 0.88 and 1.91 μ g/mL for men and 2.19 and 3.90 μ g/mL for women.

[†]Statistical tests for trend (two-sided) were assessed by assigning ordinal values to tertiles of each measurement.

[‡]Cutoff points were 1,756 and 3,842 pg/mL for men and 3,856 and 7,908 pg/mL for women.

[§]Adjusted for age, screening period, duration of fasting, sex, cigarette smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, and BMI.

^{||}Values are P interaction instead of P trend.

[¶]Cutoff points were 2.38 and 2.97 pg/mL for men and 2.22 and 2.79 pg/mL for women.

Further studies in populations with larger body sizes are thus required. Finally, the present study was based not on incident but on prevalent cases, meaning that the ORs of colorectal adenoma presented in this study did not necessarily indicate the risk of “developing” colorectal adenoma, but rather the risk of “having” colorectal adenoma at a point in time, and should therefore be interpreted with caution.

In summary, adiponectin may decrease the risk of colorectal neoplasia through mechanisms other than the indirect mechanism through insulin resistance. Taking recent evidence from basic research into account, we hypothesize that adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, and that leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. Our observations add to a growing body of evidence for the interactive effects of adiponectin and leptin in the early stage of colorectal tumorigenesis separate to their profound involvement in insulin resistance.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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