

UC Davis

UC Davis Previously Published Works

Title

Adiponectin May Modify the Risk of Barrett's Esophagus in Patients With Gastroesophageal Reflux Disease.

Permalink

<https://escholarship.org/uc/item/66z75611>

Journal

Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association, 13(13)

ISSN

1542-3565

Authors

Almers, Lucy M
Graham, James E
Havel, Peter J
et al.

Publication Date

2015-12-01

DOI

10.1016/j.cgh.2015.01.009

Peer reviewed

ALIMENTARY TRACT

Adiponectin May Modify the Risk of Barrett's Esophagus in Patients With Gastroesophageal Reflux Disease



Lucy M. Almers,* James E. Graham,^{‡,§} Peter J. Havel,^{‡,§} and Douglas A. Corley*

*Division of Research, Kaiser Permanente, Oakland, California; [‡]Department of Molecular Biosciences, School of Veterinary Medicine, [§]Department of Nutrition, University of California, Davis, California

BACKGROUND & AIMS: Abdominal obesity and increasing body mass index are risk factors for esophageal adenocarcinoma and its main precursor, Barrett's esophagus; however, there are no known biological mechanisms for these associations or regarding why only some patients with gastroesophageal reflux disease develop Barrett's esophagus. We evaluated the association between Barrett's esophagus and multimers of an adipose-associated hormone, adiponectin.

METHODS: We conducted a case-control study evaluating the associations between adiponectin (total, high-molecular-weight, and low-/medium-molecular-weight) and Barrett's esophagus within the Kaiser Permanente Northern California population. Patients with a new diagnosis of Barrett's esophagus (cases) were matched to patients with gastroesophageal reflux disease (GERD) without Barrett's esophagus and to population controls.

RESULTS: Complete serologic and epidemiologic data were available for 284 cases, 294 GERD controls, and 285 population controls. Increasing adiponectin levels were a risk factor for Barrett's esophagus among patients with GERD (total adiponectin fourth vs first quartile odds ratio [OR], 1.96; 95% confidence interval [CI], 1.17–3.27; high-molecular-weight adiponectin OR, 1.65; 95% CI, 1.00–2.73; low-/medium-molecular-weight adiponectin OR, 2.18; 95% CI, 1.33–3.56), but not compared with population controls. The associations were significantly stronger among patients reporting frequent GERD symptoms and among smokers (*P* values interaction < .01).

CONCLUSIONS: Adiponectin levels are associated positively with the risk of Barrett's esophagus among patients with GERD and among smokers, but not among population controls without GERD symptoms. Higher adiponectin concentrations either independently may contribute to the aberrant healing of esophageal injury into Barrett's esophagus or be a marker for other factors.

Keywords: Esophageal Adenocarcinoma; Adiponectin; BMI; Barrett's Esophagus; Adipokines.

See editorial on page 2273; see similar articles on pages 2241 and 2265 in this issue of *Clinical Gastroenterology and Hepatology*.

The incidence of esophageal adenocarcinoma has increased more than 500% in the United States over the past 3 decades; it accounts for more than 2% of male cancer deaths.^{1,2} Barrett's esophagus likely represents a metaplastic healing response to esophageal injury, typically from gastroesophageal reflux disease (GERD).³ Its presence increases the risk for esophageal adenocarcinoma by 30- to 40-fold.⁴ Barrett's esophagus is associated with obesity, especially abdominal obesity,^{5,6} although the biological links between obesity, Barrett's esophagus, and cancer are unclear.^{5,7,8}

Circulating adiponectin, an adiposity-associated hormone, is associated inversely with adiposity and insulin

resistance^{9,10}; it may represent a mechanistic link between Barrett's esophagus and obesity.^{11–13} Animal models suggest that adiponectin influences the healing response of the gastrointestinal mucosa.¹⁴ Mice lacking adiponectin, for example, had more ethanol-induced gastric injury than normal mice, whereas adiponectin administration improved gastric mucosal repair.¹⁴ Adiponectin is also a potentially modifiable factor, given that adiponectin agonists and homologs currently are being studied as potential therapeutic agents.^{15,16}

Abbreviations used in this paper: BMI, body mass index; CI, confidence interval; GERD, gastroesophageal reflux disease; KPNC, Kaiser Permanente Northern California; OR, odds ratio.

Most current article

© 2015 by the AGA Institute
1542-3565/\$36.00

<http://dx.doi.org/10.1016/j.cgh.2015.01.009>

We evaluated the associations between circulating adiponectin subtypes and the risk of Barrett's esophagus using 2 control groups: patients diagnosed with GERD and population controls.

Methods

Study Design and Population

We conducted a nested case-control study within Kaiser Permanente Northern California (KPNC).¹⁷ Patients were 18 to 79 years of age and members continuously for 2 or more years before their index date. The design and analyses were approved by the KPNC institutional review board (September 2002).

Case Definition

Cases were eligible KPNC members with a new diagnosis of Barrett's esophagus between October 2002 and September 2005, identified using the International Classification of Diseases, 9th revision code 530.2, defined at KPNC as "Barrett's esophagitis." A gastroenterologist (D.A.C.) reviewed the endoscopy and pathology records. Patients were included if there was a visible length of columnar-type epithelium proximal to the gastroesophageal junction/gastric folds, and an esophageal biopsy specimen showed specialized intestinal epithelium¹⁸ (after pathologist slide review). Patients were excluded if they had only gastric-type or columnar metaplasia without intestinal metaplasia, lacked an esophageal biopsy specimen or had biopsy specimens only of a mildly irregular squamocolumnar junction, had a prior diagnosis of Barrett's esophagus, or had esophageal cancer (dysplasia was included). The index date was the date of Barrett's esophagus diagnosis.

Gastroesophageal Reflux Disease Controls

GERD control members had all of the following before entry: a GERD-related International Classification of Diseases, 9th revision code (530.11 [reflux esophagitis] or 530.81 [gastroesophageal reflux]), a prescription for 90 days' or more use of a histamine-2-receptor antagonist or a proton pump inhibitor in the previous year, no prior diagnosis of Barrett's esophagus, and a recent esophagogastroduodenoscopy that did not show esophageal columnar metaplasia of any type.

Population Controls

Population controls were selected randomly from the at-risk (no prior Barrett's esophagus diagnosis) KPNC membership, using risk set sampling.¹⁹ The index date for controls was the midpoint of each 2- to 3-month case selection interval. The population and GERD controls were frequency matched to cases by sex, age at index

date (5-year age groups), and geographic region (medical facility).

Exposure Measurements

Serum samples were stored at -80°C because adiponectin is stable when frozen.^{20,21} Concentrations were assayed in duplicate, using mixed cases and controls, by researchers experienced with adiponectin analysis (P.J.H.). High-molecular-weight adiponectin measurements used an enzyme-linked immunosorbent assay (ALPCO, Salem, NH), total measurements used a radioimmunoassay (Millipore, Billerica, MA). We compared same-sample interassay values and adjusted for differences with a conversion factor. The adiponectin ratio is the high-molecular-weight adiponectin/total adiponectin.

All subjects completed the following: an in-person interview of GERD symptoms and use of medications, tobacco, and alcohol (all for the year before the index date and longer exposures); a validated food frequency questionnaire (Block et al,²² full-length questionnaire); and measurements of height, weight, waist (obtained at the iliac crest while standing) and thigh circumferences, and serum *Helicobacter pylori* serum antibody status. Examinations used trained interviewers, most commonly at the subject's home. GERD assessments used a validated symptom questionnaire²³ for heartburn or acid regurgitation.

Statistical Analysis

The study used standard techniques for unpaired case-control studies, including unconditional logistic regression.^{19,24} Comparisons of proportions used binomial distribution (Stata version 10.1; Stata Corp, College Station, TX). Continuous adiponectin measures used log-transformed values. Quartiles used distributions among the population controls and sex-specific quartiles for sex-stratified analyses.

We evaluated the following as potential confounders: education (<7 , 7-9, 10-11, 12+ y); smoking status (≥ 20 vs < 20 lifetime-packs)²⁵; alcohol (ever vs never drank alcohol); total daily calories, antioxidants (vitamins A, C, and E; carotene; and selenium), fat intake, fruits, vegetables, and iron; multivitamins; GERD symptom frequency ($<$ weekly vs \geq weekly); a comorbidity index^{26,27}; *H pylori* serum antibody status; aspirin and nonsteroidal anti-inflammatory medication use; body mass index (BMI); waist circumference; and race. We evaluated for effect modification by race, sex, smoking, and GERD symptom frequency using cross-product terms in the crude logistic regression model and stratum-specific odds ratios (ORs).²⁸

A potentially confounding variable was retained if it changed the main effect OR by 10% or more for at least 2 adiponectin variables (total adiponectin, high-molecular-weight adiponectin, low-/medium-molecular-weight

adiponectin, and the adiponectin ratio). Final models therefore were adjusted for waist circumference, race, and the main frequency-matched variables (sex and age). The covariates were evaluated separately for both population-based controls and GERD controls. The variables that changed the OR by more than 10% were similar for both comparison groups, thus the same confounding structure was used in the model for both comparisons.

Results

Patient Characteristics

Complete data were available for 96.8% (n = 923) of all interviewed subjects (Table 1). Thirty persons were excluded because of missing values for the following: waist (n = 4), serum availability for adiponectin measurement (n = 25), and race/ethnicity (n = 1). GERD controls had a lower mean weight (184.7 lb) than cases (191.5 lb) or population controls (191.8 lb). Cases were more likely to have at least weekly GERD symptoms (80.0%) (vs GERD controls [73.4%] or population controls [28.2%]) and to be non-Hispanic whites (87.4%) (vs population controls [84.9%] or GERD controls [80.5%]).

Cases Versus Gastroesophageal Reflux Disease Controls

Participants in the fourth (vs first) quartile of total and low+medium-weight adiponectin were twice as likely to have Barrett's esophagus (total adiponectin OR, 1.96; 95% confidence interval [CI], 1.17–3.27; low+medium-weight OR, 2.18; 95% CI, 1.33–3.56) (Table 2, Figure 1). Similarly, participants in the fourth quartile of high-molecular-weight adiponectin were more likely to have Barrett's esophagus (OR, 1.65; 95% CI, 1.00–2.73) (Table 2, Figure 1). Similar significant associations were seen for continuous measures of total, high-molecular-weight, and low-/medium-molecular-weight adiponectin (Table 2).

Cases Versus Population Controls

Increasing adiponectin levels were associated with Barrett's esophagus among population controls with GERD symptoms, but not among population controls overall or population controls with minimal or no GERD symptoms (*P* value interaction total adiponectin, *P* < .001) (Tables 2 and 3, Figure 2). Continuous measures of total and high-molecular-weight adiponectin were associated positively with Barrett's esophagus among cases/population controls with frequent GERD symptoms (\geq weekly), comparable with subjects in the larger physician-defined GERD control group (total adiponectin: OR, 1.68; 95% CI, 1.01–2.80; high-molecular-weight adiponectin: OR, 1.50; 95% CI,

Table 1. Characteristics of Study Groups

	Cases	Population controls	GERD controls
Subjects, n (%)	310 (100.0)	305 (100.0)	308 (100.0)
Mean age, y (\pm SD)	62 (\pm 10.7)	62 (\pm 10.2)	62 (\pm 10.7)
Age, n (%)			
20–39 y	7 (2.3)	9 (3.0)	11 (3.6)
40–59 y	118 (38.1)	102 (33.4)	111 (36.0)
60–79 y	185 (59.7)	194 (63.6)	186 (60.4)
Race, n (%)			
Non-Hispanic white	271 (87.4)	259 (84.9)	248 (80.5)
Black	4 (1.3)	16 (5.2)	20 (6.5)
Hispanic	24 (7.7)	12 (3.9)	20 (6.5)
Asian	3 (1.0)	8 (2.6)	7 (2.3)
Other	8 (2.6)	10 (3.3)	13 (4.2)
Sex, n (%)			
Male	227 (73.2)	208 (68.2)	212 (68.8)
Smoking status (ever smoked), n (%)	206 (66.5)	170 (55.7)	183 (59.4)
GERD score, ^a n (%)			
Any GERD symptoms	289 (93.2)	185 (60.7)	289 (93.8)
At least weekly	248 (80.0)	86 (28.2)	226 (73.4)
Mean current weight (\pm SD), kg	86.9 (\pm 21.4)	87.0 (\pm 18.8)	83.8 (\pm 16.8)
Mean waist (\pm SD), cm	100.7 (\pm 14.9)	99.1 (\pm 17.5)	97.2 (\pm 14.3)
BMI, ^b mean (\pm SD)	29.4 (\pm 6.1)	29.4 (\pm 5.8)	28.8 (\pm 5.2)
BMI, ^b n (%)			
Underweight	5 (1.6)	2 (0.7)	4 (1.3)
Normal	60 (19.4)	68 (22.3)	63 (20.5)
Overweight	122 (39.4)	116 (38.0)	134 (43.5)
Obese	123 (39.7)	119 (39.0)	107 (34.7)
Adiponectin multimers, mean (\pm SD)			
Total, μ g/mL	12.8 (\pm 7.7)	12.1 (\pm 6.7)	11.7 (\pm 6.8)
High molecular weight (\pm SD), μ g/mL	3.4 (\pm 2.9)	3.2 (\pm 2.6)	3.1 (\pm 2.7)
Low + medium molecular weight (\pm SD), μ g/mL	4.3 (\pm 2.0)	4.1 (\pm 1.8)	3.9 (\pm 1.7)
Ratio: high molecular weight/total (\pm SD)	0.40 (\pm 0.1)	0.40 (\pm 0.1)	0.40 (\pm 0.1)

^aGERD score represents the patient's report of frequency of GERD symptoms, which is not a criterion for GERD diagnosis. Thus, the category of "Any GERD symptoms" will not be 100% among GERD control group subjects.

^bBMI categories were based on international standards as presented by the World Health Organization Global Database on Body Mass Index.

1.04–2.15) (Table 3). Analyses by quartile had overall similar directions, although with wider confidence intervals.

Smoking

Total adiponectin. For comparisons with GERD controls, there were positive associations between total, high-molecular-weight, and low-/medium-molecular-weight adiponectin and Barrett's esophagus among ever smokers (total adiponectin: OR, 1.83; 95% CI, 1.20–2.78; high-molecular-weight adiponectin: OR, 1.46; 95% CI, 1.09–1.97; low-/medium-molecular-weight adiponectin: OR, 2.11; 95% CI, 1.27–3.49), but not among never

Table 2. Adiponectin and Barrett's Esophagus: Cases Versus Population and GERD Control Groups

	Counts			Cases vs population controls		Cases vs GERD controls	
	Cases	Population controls	GERD controls	Adjusted OR	95% CI	Adjusted OR	95% CI
Total adiponectin							
Quartiles, $\mu\text{g/mL}$							
<7.35	80	77	81	Ref		Ref	
7.36–10.55	63	76	88	0.83	(0.53–1.33)	0.79	(0.50–1.25)
10.56–15.41	82	76	75	1.13	(0.72–1.77)	1.33	(0.84–2.11)
>12.46	85	76	64	1.36	(0.83–2.23)	1.96	(1.17–3.27)
Continuous, log-transformed	310	305	308	1.36	(0.97–1.89)	1.66	(1.19–2.32)
Adiponectin high MW							
Quartiles, $\mu\text{g/mL}$							
<1.46	82	77	89	Ref		Ref	
1.46–2.53	74	76	77	0.95	(0.61–1.50)	1.16	(0.74–1.83)
2.54–4.00	69	76	66	0.92	(0.58–1.45)	1.32	(0.82–2.12)
>4.02	85	76	76	1.32	(0.80–2.17)	1.65	(1.00–2.73)
Continuous, log-transformed	310	305	308	1.20	(0.96–1.51)	1.33	(1.05–1.68)
Adiponectin low + medium MW							
Quartiles, $\mu\text{g/mL}$							
<2.75	72	77	87	Ref		Ref	
2.75–3.80	78	76	76	1.15	(0.73–1.81)	1.33	(0.84–2.10)
3.80–5.07	69	76	77	1.06	(0.66–1.69)	1.25	(0.79–1.99)
>5.07	91	76	68	1.56	(0.97–2.53)	2.18	(1.33–3.56)
Continuous, log-transformed	310	305	308	1.44	(0.98–2.11)	1.88	(1.28–2.78)
Adiponectin ratio high MW/total							
Quartiles							
<0.32	86	77	67	Ref		Ref	
0.32–0.39	67	76	93	0.81	(0.51–1.27)	0.58	(0.36–0.91)
0.39–0.47	82	76	70	1.03	(0.66–1.63)	0.92	(0.57–1.48)
>0.47	75	76	78	0.98	(0.61–1.57)	0.82	(0.50–1.34)
Continuous, log-transformed	310	305	308	1.22	(0.71–2.09)	1.19	(0.68–2.08)

NOTE. Adjusted OR was adjusted for sex, age, race/ethnicity, and waist circumference. Quartiles were based on the population control groups. MW, molecular weight.

smokers (Supplementary Table 1, Figure 3). The associations were strongest among current smokers, although with limited power to evaluate current smokers (Supplementary Table 1, Figure 3). Similar positive associations between total, high-molecular-weight, and medium-molecular-weight adiponectin and Barrett's esophagus also were seen in the case vs population control comparison group for ever smokers, but not for never smokers (data not shown).

Stratifications by Sex

The associations between total adiponectin levels and Barrett's esophagus were somewhat stronger among women than among men, among GERD controls (continuous total adiponectin women: OR, 2.27; 95% CI, 1.15–4.47; men: OR, 1.50; 95% CI, 1.02–2.22; *P* value interaction, .10) (Supplementary Table 2). Low-/medium-molecular-weight adiponectin had significant associations or strong trends with the risk of Barrett's esophagus among both men and women, whereas high-molecular-weight adiponectin showed significant associations only among women (Supplementary Table 2).

For cases vs population controls, women showed significant associations for total adiponectin, high-molecular-weight adiponectin, and adiponectin ratio, but not for low-/medium-molecular-weight adiponectin (data not shown).

Stratifications by Body Mass Index

The nonsignificant trends for associations between adiponectin and Barrett's esophagus were stronger among persons with a normal BMI (eg, log-transformed total adiponectin, continuous value OR, 2.22; 95% CI, 0.98–5.04), than among overweight (OR, 1.32; 95% CI, 0.76–2.30) or obese persons (OR, 1.15; 95% CI, 0.68–1.94). Similar patterns were seen for high- and low+medium-weight adiponectin multimers (Supplementary Table 3).

Influence of Proton Pump Inhibitors

Among the population controls, mean adiponectin levels were similar between PPI users vs nonusers (mean, 11.88 vs 12.99, respectively; *P* = .24). A logistic

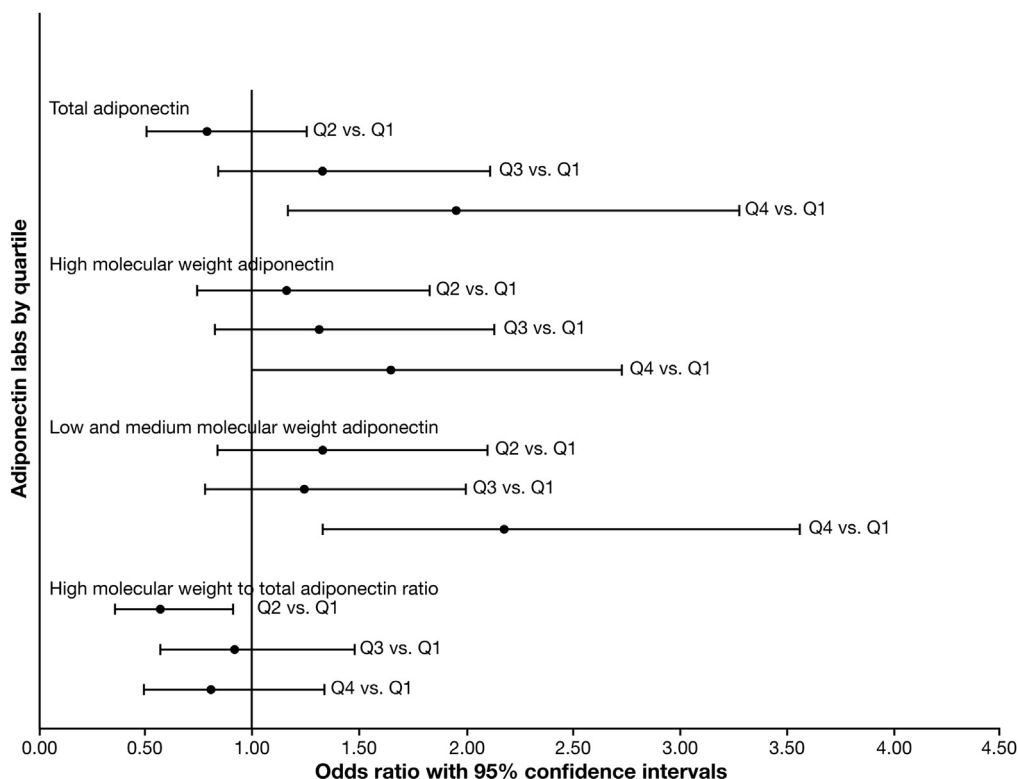


Figure 1. Adiponectin and Barrett's esophagus: cases vs GERD controls. Odds ratios (black circles) and 95% confidence intervals (black bars) for adiponectin quartiles adjusted for sex, age, race/ethnicity, and waist circumference. Q, quartile.

regression model adjusted for sex, age, and BMI showed no significant association between adiponectin levels and PPI use (data not shown).

Evaluation of Assumptions

We evaluated previously reported associations for adiponectin using the population controls.^{29–32} As expected, the mean adiponectin levels decreased with increasing BMI ($P < .0001$), were lower among men ($P < .001$), and increased with age ($P < .0001$). Unlike some prior reports, it was not correlated with smoking status (ever vs never).³²

If adiponectin mediates the association between waist circumference and Barrett's esophagus, we would expect the association between adiponectin and Barrett's esophagus to diminish after controlling for obesity. On the contrary, for cases vs GERD controls, the association for low-/medium-molecular-weight adiponectin actually strengthened after adjusting for BMI (unadjusted OR, 1.45; 95% CI, 1.01–2.08; adjusted OR, 1.55; 95% CI, 1.07–2.25), and for both BMI and waist circumference (OR, 1.80; 95% CI, 1.22–2.65).

Discussion

These results suggest that increasing serum concentrations of total adiponectin, high-molecular-weight adiponectin, and low-/medium-molecular-weight adiponectin are associated with an increased risk of Barrett's esophagus among people with a physician-assigned

GERD diagnosis, population controls with frequent self-reported GERD symptoms and smokers, but not among persons without GERD symptoms and nonsmokers.

The current study adds knowledge regarding the associations between adiponectin and the body's response to injury. Barrett's esophagus is thought to result from an aberrant healing response to esophageal injury, most commonly gastroesophageal reflux. A positive association between adiponectin and esophageal healing is biologically plausible; animal models show it is associated strongly with gastrointestinal mucosal healing after caustic injury.¹⁴ Adiponectin may modify pathways for cell injury and repair, such as the nitric oxide and interleukin pathways.^{11,12} Smoking also is associated with both the risk of esophagitis and the risk of Barrett's esophagus independent of GERD.³³ Two potential explanations for our findings include the following: higher adiponectin levels may increase the risk of a metaplastic healing response (ie, Barrett's esophagus) in response to GERD-induced injury and smoking; or higher adiponectin levels may increase the risk of esophagitis, which is associated with both smoking and GERD. The second explanation is less likely given that adiponectin was not associated independently with either smoking or GERD symptoms among our population controls (data not shown).

Adiponectin's associations with mucosal healing may differ from its other roles with carcinogenesis. Adiponectin levels have been associated inversely with the risk of prostate, colon, gastric, endometrial, and breast cancers.^{34–38} Adiponectin inhibits leptin-induced cell growth,³⁹ suggesting that high serum concentrations

Table 3. Associations of Adiponectin and Barrett's Esophagus: Stratified by GERD Symptom Frequency for Cases Versus Population Controls

	Less than weekly ^a				At least weekly ^b			
	Cases	Controls ^c	Adjusted OR	95% CI	Cases	Controls	Adjusted OR	95% CI
Total adiponectin interaction term	<0.001							
Quartiles, $\mu\text{g/mL}$								
<7.35	19	51	Ref		61	26	Ref	
7.36–10.55	14	51	0.67	(0.29–1.55)	49	25	0.83	(0.42–1.64)
10.56–15.41	9	60	0.39	(0.16–0.99)	73	15	2.11	(1.00–4.45)
>15.46	20	56	1.24	(0.52–2.95)	65	20	1.56	(0.72–3.37)
Continuous, log-transformed	62	218	1.16	(0.62–2.20)	248	86	1.68	(1.01–2.80)
Adiponectin high MW interaction term	<0.001							
Quartiles, $\mu\text{g/mL}$								
<1.46	20	48	Ref		62	29	Ref	
1.46–2.53	14	53	0.55	(0.24–1.27)	60	23	1.18	(0.60–2.32)
2.54–4.00	12	61	0.42	(0.18–0.99)	57	15	1.72	(0.82–3.63)
>4.02	16	56	0.84	(0.35–2.04)	69	19	1.95	(0.89–4.29)
Continuous, log-transformed	62	218	0.98	(0.64–1.51)	248	86	1.50	(1.04–2.15)
Adiponectin low + medium MW interaction term	<0.001							
Quartiles, $\mu\text{g/mL}$								
<2.75	16	56	Ref		56	21	Ref	
2.75–3.80	16	52	1.02	(0.45–2.33)	62	24	0.91	(0.45–1.85)
3.80–5.07	12	55	0.80	(0.33–1.93)	57	20	1.16	(0.55–2.43)
>5.07	18	55	1.57	(0.66–3.72)	73	21	1.33	(0.62–2.84)
Continuous, log-transformed	62	218	1.52	(0.72–3.21)	248	86	1.56	(0.87–2.80)
Adiponectin ratio high MW/total interaction term	<0.001							
Quartiles								
<0.32	19	48	Ref		67	29	Ref	
0.32–0.39	12	52	0.56	(0.24–1.33)	55	24	0.95	(0.48–1.86)
0.39–0.47	16	60	0.70	(0.31–1.58)	66	15	1.95	(0.92–4.11)
>0.47	15	58	0.73	(0.32–1.69)	60	18	1.49	(0.70–3.17)
Continuous, log-transformed	62	218	0.66	(0.26–1.67)	248	86	2.27	(0.96–5.41)

NOTE. Adjusted OR was adjusted for sex, age, race/ethnicity, and waist circumference. Quartiles were based on the population control groups.

^aReported frequency of GERD symptoms as less than weekly.

^bReported frequency of GERD symptoms as weekly or more than weekly.

^cNot all population controls had GERD frequency data.

may protect against cancer. A study by Yildirim et al⁴⁰ found lower levels of adiponectin among patients with esophageal cancer (n = 75 cases, 13 with adenocarcinoma). Studies in cancer patients, however, have difficulty excluding changes in adiponectin caused by the cancer, such as alteration of diet, exercise, or metabolism. Another study, which looked at the relationship between adiponectin-receptor expression in esophageal adenocarcinoma cancer cells,⁴¹ found that greater amounts of visceral fat were associated with greater expression of the adiponectin receptor-2, independent of serum adiponectin levels.⁴¹

The current results differ from 2 of 3 prior smaller studies of Barrett's esophagus. One study found lower levels of low-molecular-weight serum adiponectin among 112 Barrett's esophagus patients vs GERD control patients (third vs first tertiles: OR, 0.33; 95% CI, 0.16–0.69) and no significant associations for total or high-molecular-weight adiponectin; the study did not

include population controls.⁴² The second study found lower total adiponectin levels among 177 Barrett's esophagus cases vs population controls (third vs first tertiles: OR, 0.56; 95% CI, 0.32–0.98).⁴³ In contrast, the third study found a nonsignificant trend for a positive association between adiponectin and Barrett's esophagus, and significant positive associations among white males (fourth vs first quartiles: OR, 3.27; 95% CI, 1.06–10.05).⁴⁴ We cannot fully account for the differences between the prior studies and the current findings, although some groups of patients without GERD in our study did have inverse associations (Table 3) and some of the other studies adjusted for smoking, GERD, and hiatal hernia whereas we evaluated these primarily as sources of interaction. All 3 prior studies had much smaller sample sizes (decreasing the power to look for interaction), slightly younger populations, and some had fewer smokers in the control groups.^{41–44} Similar to the current study, Thompson et al included only new

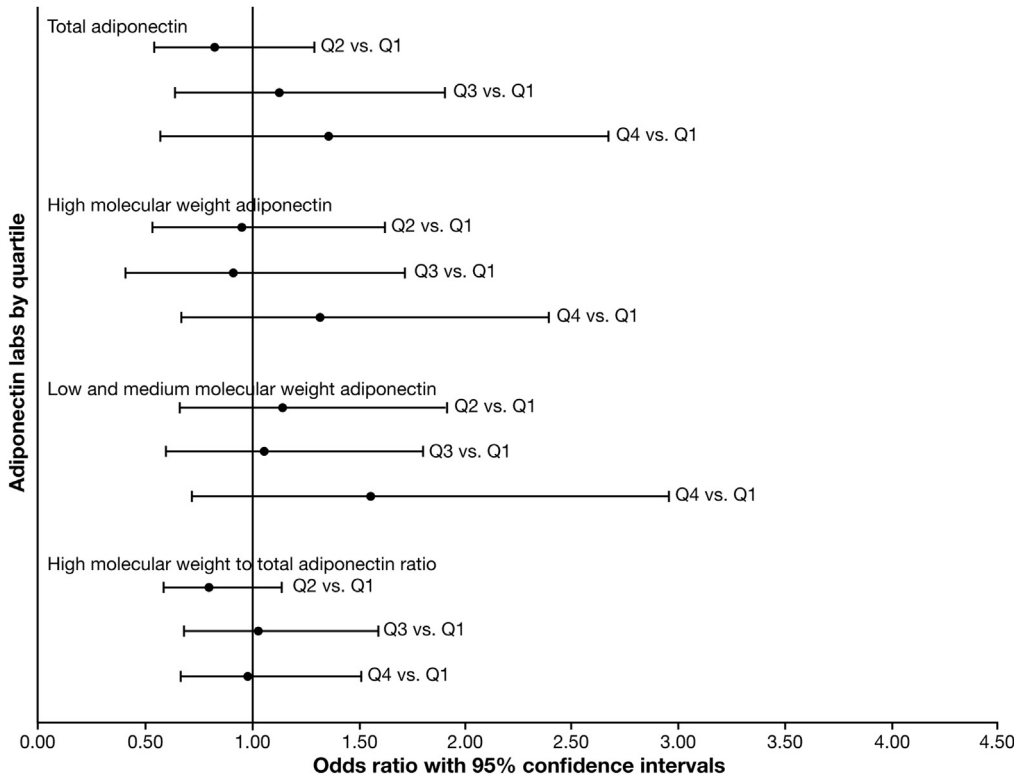


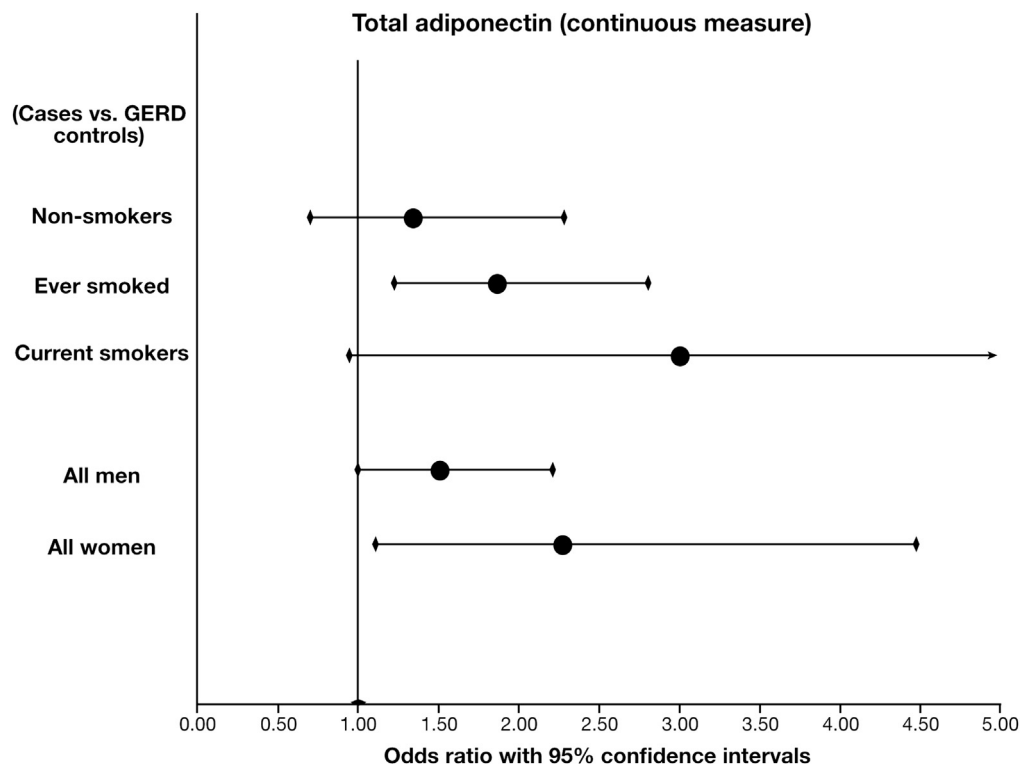
Figure 2. Adiponectin and Barrett’s esophagus: cases vs population controls. Odds ratios (black circles) and 95% confidence intervals (black bars) for adiponectin quartiles adjusted for sex, age, race/ethnicity, and waist circumference. Q, quartile.

diagnoses of Barrett’s esophagus, whereas Rubenstein et al included both prevalent and new diagnoses and reported somewhat lower mean adiponectin levels.^{42,43}

The current results run counter to the general inverse associations between adiponectin levels and obesity, and the positive associations between abdominal obesity and

Barrett’s esophagus.^{5,45,46} However, prior positive associations between abdominal obesity and Barrett’s esophagus were observed mainly in comparisons with population-based controls; no strong associations were found among patients with GERD.⁵ Thus, given the known associations between adiponectin and mucosal

Figure 3. Adiponectin and Barrett’s esophagus: cases vs GERD controls, stratified by smoking status and sex. Stratified odds ratios (black circles) and 95% confidence intervals (black bars) for adiponectin log-transformed concentrations adjusted for age, race/ethnicity, and waist circumference.



healing, our current results represent a potential mechanism whereby only some patients with GERD develop Barrett's esophagus. The associations between abdominal obesity and Barrett's esophagus among population controls may not be mediated strongly by adiponectin.

There were several strengths of the current study. First, positive associations between adiponectin and Barrett's esophagus were found in both population controls with self-reported GERD symptoms and patients with physician-assigned GERD diagnoses. Second, our analyses showed the expected associations between adiponectin levels and sex, age, and BMI; this also makes bias in the laboratory or with patient sampling less likely. Third, we studied a large group of patients with a new diagnosis of Barrett's esophagus within a community-based population, thereby minimizing selection bias found in persons with prevalent Barrett's esophagus. Fourth, the number of cases was large,⁴³ providing greater power to evaluate interactions. Fifth, the 2 control groups allowed a separate evaluation of why only some GERD patients develop Barrett's esophagus. Sixth, the data were of high quality, with validated questionnaires, detailed anthropometric measurements, direct review of the endoscopy reports, and manual pathology slide review.

The analyses had several potential limitations. First, case-control studies cannot establish cause and effect; adiponectin levels may differ without it, causing Barrett's esophagus.¹⁹ We cannot exclude incomplete control of confounding. Second, we did not measure the adiponectin levels at exactly the time the Barrett's esophagus developed, given that time is unknown. Fourth, low- and medium-molecular-weight adiponectin were calculated through subtraction of measured high-molecular-weight adiponectin. Although the low- and medium-molecular-weight forms of adiponectin together are the predominant forms in the circulation,⁴⁷ high-molecular-weight adiponectin appears to be the most bioactive form in terms of regulating glucose homeostasis and insulin sensitivity.¹⁰ The finding of a borderline stronger association between adiponectin and Barrett's esophagus among women vs men is interesting given that women are at lower risk than men for this condition. Although adiponectin levels are known to differ between men and women, the interaction term was of marginal statistical significance ($P = .10$) and there may be sex-specific differences in its biological activities.

In summary, in a community-based population, there was an association between increasing levels of serum adiponectin multimers and a new diagnosis of Barrett's esophagus among patients with GERD. This association was stronger among smokers, and among persons with more frequent GERD. These results suggest a potential for a direct mechanistic role for adiponectin in the process of mucosal healing that may cause Barrett's esophagus, although it also is possible that circulating adiponectin concentrations are a marker for another process, such as systemic inflammation related to

Barrett's esophagus. Although the results run counter to the expected direction of obesity's general associations with Barrett's esophagus (and with adiponectin levels), they represent one of the first potential biological risk factors identified for why only some patients with GERD and smoking develop Barrett's esophagus.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at <http://dx.doi.org/10.1016/j.cgh.2015.01.009>.

References

1. Blot WJ, McLaughlin JK. The changing epidemiology of esophageal cancer. *Semin Oncol* 1999;26:2–8.
2. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005;97:142–146.
3. Reynolds RP. Pathophysiology and investigation of Barrett's esophagus. *Can J Gastroenterol* 1997;11(Suppl B):41B–44B.
4. Sikkema M, de Jonge PJ, Steyerberg EW, et al. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010;8:235–244; quiz e32.
5. Corley DA, Kubo A, Levin TR, et al. Abdominal obesity and body mass index as risk factors for Barrett's esophagus. *Gastroenterology* 2007;133:34–41; quiz 311.
6. Murray L, Romero Y. Role of obesity in Barrett's esophagus and cancer. *Surg Oncol Clin N Am* 2009;18:439–452.
7. Samanic C, Gridley G, Chow WH, et al. Obesity and cancer risk among white and black United States veterans. *Cancer Causes Control* 2004;15:35–43.
8. Sharma P, Falk GW, Weston AP, et al. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2006;4:566–572.
9. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 2004;53(Suppl 1):S143–S151.
10. Swarbrick MM, Havel PJ. Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. *Metab Syndr Relat Dis* 2008;6:87–102.
11. Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R1220–R1225.
12. Chen H, Montagnani M, Funahashi T, et al. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003;278:45021–45026.
13. Corley DA. Obesity and the rising incidence of oesophageal and gastric adenocarcinoma: what is the link? *Gut* 2007;56:1493–1494.
14. Yamamoto S, Watabe K, Araki H, et al. Protective role of adiponectin against ethanol-induced gastric injury in mice. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G773–G780.
15. Kupchak BR, Garitaonandia I, Villa NY, et al. Antagonism of human adiponectin receptors and their membrane progesterone receptor paralogs by TNFalpha and a ceramidase inhibitor. *Biochemistry* 2009;48:5504–5506.

16. Miele M, Costantini S, Colonna G. Structural and functional similarities between osmotin from *Nicotiana tabacum* seeds and human adiponectin. *PLoS One* 2011;6:e16690.
17. Krieger N. Overcoming the absence of socioeconomic data in medical records: validation and application of a census-based methodology. *Am J Public Health* 1992;82:703–710.
18. Sharma P, McQuaid K, Dent J, et al. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. *Gastroenterology* 2004;127:310–330.
19. Rothman KJ. *Modern epidemiology*. Philadelphia: Lippincott-Raven, 1998.
20. Choi KM, Lee J, Lee KW, et al. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. *Clin Endocrinol* 2004; 61:75–80.
21. Pischon T, Hotamisligil GS, Rimm EB. Adiponectin: stability in plasma over 36 hours and within-person variation over 1 year. *Clin Chem* 2003;49:650–652.
22. Block G, Thompson FE, Hartman AM, et al. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. *J Am Diet Assoc* 1992; 92:686–693.
23. Locke GR, Talley NJ, Weaver AL, et al. A new questionnaire for gastroesophageal reflux disease. *Mayo Clin Proc* 1994; 69:539–547.
24. Breslow NE. *Statistical methods in cancer research. Volume 1: the analysis of case-control studies*. Lyon, France: International Agency for Research on Cancer, 1980.
25. Ferris BG. *Epidemiology Standardization Project* (American Thoracic Society). *Am Rev Respir Dis* 1978;118:1–120.
26. Zhao Y, Ash AS, Ellis RP, et al. Predicting pharmacy costs and other medical costs using diagnoses and drug claims. *Med Care* 2005;43:34–43.
27. Zhao Y, Ellis RP, Ash AS, et al. Measuring population health risks using inpatient diagnoses and outpatient pharmacy data. *Health Serv Res* 2001;36:180–193.
28. Hosmer DW. *Applied logistic regression*. New York: Wiley, 2000.
29. Isobe T, Saitoh S, Takagi S, et al. Influence of gender, age and renal function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol* 2005;153:91–98.
30. Kern PA, Di Gregorio GB, Lu T, et al. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- α expression. *Diabetes* 2003;52:1779–1785.
31. Nishizawa H, Shimomura I, Kishida K, et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes* 2002;51:2734–2741.
32. Takefuji S, Yatsuya H, Tamakoshi K, et al. Smoking status and adiponectin in healthy Japanese men and women. *Prev Med* 2007;45:471–475.
33. Cook MB, Shaheen NJ, Anderson LA, et al. Cigarette smoking increases risk of Barrett's esophagus: an analysis of the Barrett's and Esophageal Adenocarcinoma Consortium. *Gastroenterology* 2012;142:744–753.
34. Goktas S, Yilmaz MI, Caglar K, et al. Prostate cancer and adiponectin. *Urology* 2005;65:1168–1172.
35. Ishikawa M, Kitayama J, Kazama S, et al. Plasma adiponectin and gastric cancer. *Clin Cancer Res* 2005;11:466–472.
36. Mantzoros C, Petridou E, Dessypris N, et al. Adiponectin and breast cancer risk. *J Clin Endocrinol Metab* 2004;89: 1102–1107.
37. Petridou E, Mantzoros C, Dessypris N, et al. Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. *J Clin Endocrinol Metab* 2003;88: 993–997.
38. Wei EK, Giovannucci E, Fuchs CS, et al. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. *J Natl Cancer Inst* 2005;97:1688–1694.
39. Ogunwobi OO, Beales IL. Globular adiponectin, acting via adiponectin receptor-1, inhibits leptin-stimulated oesophageal adenocarcinoma cell proliferation. *Mol Cell Endocrinol* 2008; 285:43–50.
40. Yildirim A, Bilici M, Cayir K, et al. Serum adiponectin levels in patients with esophageal cancer. *Jpn J Clin Oncol* 2009; 39:92–96.
41. Howard JM, Beddy P, Ennis D, et al. Associations between leptin and adiponectin receptor upregulation, visceral obesity and tumour stage in oesophageal and junctional adenocarcinoma. *Br J Surg* 2010;97:1020–1027.
42. Rubenstein JH, Kao JY, Madanick RD, et al. Association of adiponectin multimers with Barrett's oesophagus. *Gut* 2009; 58:1583–1589.
43. Thompson OM, Beresford SA, Kirk EA, et al. Serum leptin and adiponectin levels and risk of Barrett's esophagus and intestinal metaplasia of the gastroesophageal junction. *Obesity (Silver Spring)* 2010;18:2204–2211.
44. Garcia JM, Splenser AE, Kramer J, et al. Circulating inflammatory cytokines and adipokines are associated with increased risk of Barrett's esophagus: a case-control study. *Clin Gastroenterol Hepatol* 2014;12:229–238 e3.
45. Kramer JR, Fischbach LA, Richardson P, et al. Waist-to-hip ratio, but not body mass index, is associated with an increased risk of Barrett's esophagus in white men. *Clin Gastroenterol Hepatol* 2013;11:373–381 e1.
46. Kubo A, Cook MB, Shaheen NJ, et al. Sex-specific associations between body mass index, waist circumference and the risk of Barrett's oesophagus: a pooled analysis from the international BEACON consortium. *Gut* 2013;62:1684–1691.
47. Pajvani UB, Hawkins M, Combs TP, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004;279:12152–12162.

Reprint requests

Address requests for reprints to: Douglas Corley, MD, PhD, MPH, Division of Research, Kaiser Permanente, 2000 Broadway, Oakland, California 94612. e-mail: Douglas.Corley@kp.org; fax: (510) 891-3802.

Conflicts of interest

The authors disclose no conflicts.

Funding

Supported by the National Institute of Diabetes and Digestive and Kidney Disease (R56 DK087748 and RO1 DK63616 [D.C.]).

Supplementary Table 1. Effects of Adipokines on Barrett's Esophagus: Stratified by Never, Ever, Former, and Current Smokers for Cases Versus GERD Controls

	Never smoker			Ever smoker			Former smoker			Current smoker		
	Cases/ controls, n	Adjusted OR	95% CI	Cases/ controls, n	Adjusted OR	95% CI	Cases/ controls, n	Adjusted OR	95% CI	Cases/ controls, n	Adjusted OR	95% CI
N		217			361			297			64	
Total adiponectin												
Interaction term	0.247 (never vs ever)						0.161 (former vs current)					
Quartiles $\mu\text{g/mL}$												
<7.35	32/29	Ref		48/52	Ref		42/43	Ref		6/9	Ref	
7.36–10.55	18/41	0.41	0.19–0.87	44/47	1.16	0.65–2.07	37/41	1.05	0.56–1.97	7/6	2.65	0.51–13.77
10.56–15.41	25/35	0.65	0.31–1.38	57/40	2.05	1.13–3.73	42/32	1.85	0.95–3.60	15/8	4.52	0.99–20.59
>15.46	28/20	1.40	0.57–3.44	57/44	2.34	1.24–4.41	44/36	2.13	1.06–4.28	13/8	4.59	0.79–26.56
Continuous, log-transformed		1.30	0.74–2.27		1.83	1.20–2.78		1.72	1.08–2.74		3.03	0.96–9.60
Adiponectin high MW												
Interaction term	0.349 (never vs ever)						0.114 (former vs current)					
Quartiles $\mu\text{g/mL}$												
<1.46	30/34	Ref		52/55	Ref		46/45	Ref		6/10	Ref	
1.46–2.53	24/36	0.80	0.38–1.66	49/41	1.47	0.82–2.62	40/35	1.31	0.70–2.46	9/6	3.54	0.71–17.62
2.54–4.00	19/30	0.69	0.31–1.54	50/36	1.89	1.03–3.46	36/30	1.59	0.81–3.12	14/6	5.26	1.15–24.09
>4.02	30/25	1.38	0.58–3.28	55/51	1.78	0.96–3.30	43/42	1.61	0.82–3.16	12/9	3.29	0.57–19.20
Continuous, log-transformed		1.06	0.71–1.58		1.46	1.09–1.97		1.39	1.01–1.92		2.22	0.96–5.14
Adiponectin low + medium MW												
Interaction term	0.167 (never vs ever)						0.300 (former vs current)					
Quartiles $\mu\text{g/mL}$												
<2.75	32/33	Ref		40/54	Ref		34/45	Ref		6/9	Ref	
2.75–3.80	24/35	0.69	0.33–1.44	54/41	1.97	1.09–3.57	42/34	1.87	0.97–3.60	12/7	2.80	0.65–12.03
3.80–5.07	16/36	0.50	0.23–1.09	52/41	2.05	1.13–3.73	44/34	2.09	1.09–4.03	8/7	2.18	0.44–10.71
>5.07	31/21	1.56	0.68–3.55	60/47	2.62	1.41–4.89	45/39	2.40	1.20–4.78	15/8	4.32	0.88–21.15
Continuous, log-transformed		1.43	0.76–2.69		2.11	1.27–3.49		2.00	1.14–3.50		3.30	0.85–12.85
Adiponectin ratio high MW/total												
Interaction term	0.064 (never vs ever)						0.244 (former vs current)					
Quartiles												
<0.32	30/26	Ref		55/41	Ref		51/35	Ref		4/6	Ref	
0.32–0.39	21/38	0.40	0.18–0.88	46/55	0.68	0.38–1.22	34/43	0.61	0.32–1.15	12/12	1.98	0.39–10.04
0.39–0.47	28/30	0.63	0.28–1.43	54/40	1.12	0.61–2.05	42/31	1.05	0.54–2.02	12/9	2.09	0.36–12.16
>0.47	24/31	0.52	0.22–1.21	51/47	1.05	0.56–1.94	38/43	0.82	0.42–1.61	13/4	5.03	0.81–31.16
Continuous, log-transformed		0.66	0.25–1.71		1.64	0.80–3.36		1.46	0.68–3.13		4.62	0.50–42.89

NOTE. Adjusted OR was adjusted for sex, age, race/ethnicity and waist circumference. Quartiles were based on the population control groups.

Supplementary Table 2. Effects of Adipokines on Barrett's Esophagus: Stratified by Sex for Cases Versus GERD Controls

	Males				Women			
	Quartiles	N Cases/ controls, n	Adjusted OR	95% CI	Quartiles	N Cases/ controls, n	Adjusted OR	95% CI
Total adiponectin								
Interaction term	0.104							
Quartiles $\mu\text{g/mL}$								
1	<6.57	55/53	Ref		<8.88	20/24	Ref	
2	6.57–8.80	44/53	0.76	0.45–1.29	8.90–12.72	12/24	0.86	0.36–2.07
3	8.82–12.90	57/53	1.17	0.66–2.05	12.73–18.22	19/24	1.65	0.69–3.93
4	>12.90	71/53	1.56	0.89–2.74	>18.37	32/24	2.96	1.10–7.97
Continuous, log-transformed			1.50	1.02–2.22			2.27	1.15–4.47
Adiponectin high MW								
Interaction term	0.230							
Quartiles $\mu\text{g/mL}$								
1	<1.25	54/53	Ref		<1.96	18/24	Ref	
2	1.26–1.96	57/53	0.97	0.57–1.64	1.96–3.12	15/24	0.78	0.32–1.89
3	1.96–3.31	49/53	1.46	0.83–2.56	3.12–4.87	17/24	1.40	0.57–3.44
4	>3.31	67/53	1.42	0.79–2.53	>4.87	33/24	2.67	1.02–7.00
Continuous, log-transformed			1.21	0.92–1.59			1.78	1.10–2.89
Adiponectin low + medium MW								
Interaction term	0.062							
Quartiles $\mu\text{g/mL}$								
1	<2.55	53/53	Ref		<3.19	17/24	Ref	
2	3.57–3.28	45/53	0.87	0.50–1.51	3.20–4.33	20/24	0.99	0.43–2.28
3	3.29–4.41	51/53	1.09	0.63–1.88	4.34–5.78	18/24	1.03	0.45–2.37
4	>4.42	78/53	2.20	1.24–3.92	>5.80	28/24	2.02	0.82–4.94
Continuous, log-transformed			1.78	1.13–2.80			2.23	1.00–4.97
Adiponectin ratio high MW/total								
Interaction term	0.371							
Quartiles								
1	<0.32	75/53	Ref		<0.35	19/24	Ref	
2	0.32–0.37	33/53	0.74	0.43–1.27	0.35–4.20	20/24	1.12	0.46–2.74
3	0.37–0.46	72/53	1.13	0.64–2.00	4.20–0.49	16/24	0.95	0.40–2.28
4	>0.46	47/53	0.75	0.41–1.35	>0.49	28/24	1.73	0.73–4.14
Continuous, log-transformed			0.93	0.49–1.78			2.65	0.81–8.59

NOTE. Adjusted OR was adjusted for sex, age, race/ethnicity, and waist circumference. Quartiles were based on the population control groups.

Supplementary Table 3. Associations of Adipokines on Barrett's Esophagus: Stratified by BMI Category for Cases Versus Population Controls

	Quartile range	Normal BMI (18.5–24.9)			Overweight BMI (25–29.0)			Obese BMI (>30)		
		Cases/controls, n	Adjusted OR	95% CI	Cases/controls, n	Adjusted OR	95% CI	Cases/controls, n	Adjusted OR	95% CI
Total adiponectin										
Interaction term			0.73			0.82			0.95	
Quartiles $\mu\text{g/mL}$										
1	<7.35	4/6			35/33			42/38		
2	7.36–10.55	6/19	0.38	0.07–1.92	28/25	1.13	0.54–2.35	28/32	0.82	0.41–1.64
3	10.56–15.41	16/12	1.81	0.39–8.40	34/34	0.96	0.48–1.93	32/30	1.04	0.52–2.07
4	>12.46	34/31	1.43	0.33–6.25	27/24	1.17	0.53–2.61	22/20	1.37	0.60–3.11
Continuous, log-transformed		60/68	2.22	0.98–5.04	124/116	1.32	0.76–2.30	124/120	1.15	0.68–1.94
Adiponectin high MW										
Interaction term			0.89			0.46			0.99	
Quartiles $\mu\text{g/mL}$										
1	<1.46	4/5			33/31			47/41		
2	1.46–2.53	8/18	0.51	0.10–2.62	36/27	1.28	0.63–2.61	30/30	0.91	0.46–1.78
3	2.54–4.00	15/13	1.46	0.30–7.12	28/33	0.84	0.41–1.73	24/31	0.74	0.37–1.50
4	>4.02	33/32	1.23	0.27–5.56	27/25	1.12	0.50–2.51	23/18	1.60	0.69–3.71
Continuous, log-transformed		60/68	1.63	0.90–2.96	124/116	1.08	0.74–1.58	124/120	1.18	0.82–1.71
Adiponectin low + medium MW										
Interaction term			0.08			0.13			0.82	
Quartiles $\mu\text{g/mL}$										
1	<2.75	4/10			29/32			37/35		
2	2.75–3.80	9/14	1.91	0.43–8.45	35/29	1.38	0.67–2.84	35/33	1.04	0.52–2.07
3	3.80–5.07	15/16	2.86	0.69–11.84	30/31	1.07	0.51–2.25	25/29	0.93	0.44–1.92
4	>5.07	32/28	3.92	0.99–15.57	30/24	1.50	0.68–3.28	27/23	1.38	0.63–3.02
Continuous, log-transformed		60/68	2.83	1.06–7.57	124/116	1.46	0.77–2.74	124/120	1.12	0.62–2.05
Adiponectin ratio high MW/total										
Interaction term			0.04			0.27			0.17	
Quartiles										
1	<0.32	5/8			41/29			42/39		
2	0.32–0.39	10/12	1.41	0.32–6.21	25/32	0.52	0.25–1.08	33/32	0.95	0.48–1.88
3	0.39–0.47	17/24	1.11	0.28–4.38	34/26	1.03	0.50–2.14	29/27	1.08	0.53–2.22
4	>0.47	28/24	1.74	0.44–6.86	24/29	0.63	0.30–1.33	20/22	0.97	0.44–2.16
Continuous, log-transformed		60/68	1.64	0.33–8.14	124/116	0.78	0.34–1.82	124/120	1.71	0.74–3.93