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Associations of Diabetes Mellitus, Insulin, Leptin, and Ghrelin With Gastroesophageal Reflux and Barrett's Esophagus

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Abstract

Background & Aims—Insulin and leptin have proliferative and anti-apoptotic effects. Ghrelin promotes gastric emptying and secretion of growth hormone and inhibits inflammation. We assessed whether diabetes mellitus and serum levels of insulin, leptin, and ghrelin are associated with gastroesophageal reflux disease (GERD) and Barrett's esophagus.

Methods—We conducted a case-control study in 822 men undergoing colorectal cancer screening who were recruited to also undergo upper endoscopy. We identified 70 with Barrett's esophagus; 80 additional men with Barrett's esophagus were recruited shortly after their clinical diagnoses. Serum levels of insulin, leptin, and ghrelin were assayed in all 104 fasting men with Barrett's esophagus without diabetes and 271 without diabetes or Barrett's esophagus. Logistic regression was used to estimate the effects of diabetes and levels of insulin, leptin, and ghrelin on GERD and Barrett's esophagus.

Results—Among men with GERD, diabetes was inversely associated with Barrett's esophagus (adjusted odds ratio [OR] = 0.383; 95% confidence interval [CI]: 0.179–0.821). Among

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nondiabetics, hyperinsulinemia was positively associated with Barrett's esophagus, but the association was attenuated by adjustment for leptin and ghrelin. Leptin was positively associated with Barrett's esophagus, adjusting for obesity, GERD, and levels of insulin and ghrelin (OR for 3rd vs 1st tertile = 3.25; 95% CI: 1.29–8.17); this association was stronger in men with GERD ($P = .01$ for OR heterogeneity). Ghrelin was positively associated with Barrett's esophagus (OR for an increment of 400 pg/mL = 1.39; 95% CI: 1.09–1.76), but inversely associated with GERD (OR for 3rd vs 1st tertile = 0.364; 95% CI: 0.195–0.680).

Conclusions—Based on a case-control study, leptin was associated with Barrett's esophagus, particularly in men with GERD. Serum insulin level was associated with Barrett's esophagus, but might be mediated by leptin. Serum ghrelin was inversely associated with GERD, as hypothesized, but positively associated with Barrett's esophagus, contrary to our hypothesis. Additional studies are needed in men and women to replicate these findings.

Keywords

Insulin; Leptin; Ghrelin; Gastroesophageal Reflux

Abdominal obesity promotes gastroesophageal reflux disease (GERD),^{1,2} a major risk factor for esophageal adenocarcinoma and the associated premalignant lesion of Barrett's esophagus (BE).^{3,4} Additionally, because obesity is associated with a number of other cancers with no known mechanical explanation,⁵ there may be additional effects that promote BE and esophageal adenocarcinoma. Abdominal obesity is positively associated with hyperinsulinemia, peripheral resistance to the actions of insulin, hyperglycemia, and diabetes mellitus. A recent genome-wide association study of BE identified an association with FOXP1, a forkhead family transcription factor.⁶ Forkhead transcription factors have been implicated in insulin sensitivity.⁷ Hyperinsulinemia has been associated with a number of epithelial cancers,^{8–11} Yet the effects of hyperinsulinemia on the risks of Barrett's esophagus and esophageal adenocarcinoma remain unclear, with inconsistent results in earlier studies.^{12–17}

Leptin is a peptide secreted primarily by adipocytes that signals satiety to the brain. Most obese humans are resistant to this signal and therefore have elevated blood levels. Leptin stimulates cell proliferation and inhibits apoptosis in esophageal adenocarcinoma cell lines via activation of the epidermal growth factor receptor in a manner that is synergistic with acid exposure.^{18,19} In 2 earlier studies, elevated circulating leptin has been associated with BE in men, but 1 study did not adjust for abdominal obesity, and the other did not adjust for GERD.^{20,21} In addition, because leptin levels are associated with insulin resistance, the effect of leptin might be confounded by insulin.

Ghrelin is a hormone secreted by the gastric fundus; it has multiple actions, including stimulating appetite, promotion of gastric emptying, enhancing growth hormone secretion, and potentially modifying inflammatory pathways, such as tumor necrosis factor- α .^{22–25} Paradoxically, obese people tend to have lower levels of circulating ghrelin.²⁶ *Helicobacter pylori* gastritis is associated with diminished gastric ghrelin production and circulating ghrelin.²⁷

The purpose of this study was to assess the possible effects of diabetes mellitus, insulin, leptin, and ghrelin on the occurrence of BE in a group of men with newly diagnosed BE and men without BE undergoing colorectal cancer (CRC) screening. We hypothesized that diabetes, insulin, and leptin would be positively associated with BE, but not with GERD or erosive esophagitis. We also hypothesized that ghrelin would be inversely associated with GERD, erosive esophagitis, and BE (Supplementary Table 1).

Methods

Study Design

We conducted a case-control study of 822 male CRC screenees, aged 50–79 years, presenting for colonoscopy at either the University of Michigan Health System's East Ann Arbor Medical Procedures Center or the Ann Arbor Veterans Affairs Medical Center. We recruited the CRC screenees to undergo upper endoscopy regardless of GERD symptoms, and we prospectively classified them on the basis of BE. BE was identified in 70 men (8.5%). In addition, we recruited 80 men aged 50–79 years who had recently been diagnosed for the first time with BE by a clinically indicated upper endoscopy at either of the 2 medical centers. The details of the methods have been reported previously.^{28,29} The study was approved by the Institutional Review Boards of the University of Michigan and the Ann Arbor Veterans Affairs Medical Center.

Anthropomorphic measurements were obtained using techniques described previously.^{30–32} CRC screenees answered questions about GERD symptoms and acid-reducing medication use before undergoing endoscopy. Patients were classified as having symptomatic GERD if they reported heartburn or regurgitation at least weekly when not taking proton pump inhibitors (PPIs) or histamine-2 receptor antagonists. GERD were further classified on the basis of whether they had taken PPIs or histamine-2 receptor antagonists and, if so, whether they reported continued symptoms at least weekly when taking those medications. If BE was suspected by the endoscopist, biopsies were obtained for review by an expert pathologist (HA). BE was defined as endoscopic suspicion of columnar mucosa proximal to the gastroesophageal junction with pathology finding of specialized intestinal metaplasia. Esophagitis was graded endoscopically by the Los Angeles (LA) Classification; patients with class C or D esophagitis were instructed to repeat the endoscopy when taking a PPI, and BE status was determined from the repeat endoscopy.³³ Patients with BE identified on a clinically indicated upper endoscopy fulfilled the same criteria for diagnosis. Electronic medical records were abstracted regarding diabetes mellitus history. A questionnaire including queries about tobacco use was typically completed after the endoscopy. Assay details can be found in the Supplementary Material.

Statistical Analysis

We conducted 2 sets of case-control analyses: 1 to estimate the effects of diabetes, insulin, leptin, and ghrelin on GERD (defined by either symptoms or erosive esophagitis) and 1 to estimate their effects on BE. For the outcomes of GERD, we selected cases of men among CRC screenees with at least weekly GERD symptoms before the use of acid-reducing medication or men found to have at least LA class B esophagitis; controls were CRC screenees without GERD symptoms, esophagitis, or BE. For outcomes of BE, we included cases of BE identified among both the CRC screenees and the men diagnosed by clinically indicated upper endoscopies. Controls were all men with at least weekly GERD symptoms before use of acid-reducing medications or men found to have at least LA class B esophagitis on the upper endoscopy, and a random selection of CRC screenees without any of those conditions.

To estimate the GERD-specific effects of diabetes, insulin, leptin, and ghrelin, we fitted logistic models to men with GERD (either weekly symptoms without any prior use of acid-reducing medications or their reported frequency before use of such medications, or at least LA class B esophagitis) and to men without GERD (defined as infrequent or no GERD symptoms and no esophagitis or only LA class A esophagitis). We used logistic regression to estimate the odds ratio for the effect of diabetes mellitus on GERD in the total sample. For the analysis of effects of insulin and leptin, we excluded subjects who were not fasting

at least 8 hours at the time of blood collection. Because diabetic medications alter serum levels of insulin, we also excluded all patients with a history of diabetes mellitus from analysis of insulin, leptin, and ghrelin effects. CRC screenees without diabetes mellitus or BE were categorized into tertiles of insulin, leptin, and ghrelin. Among the fasting nondiabetic cases of GERD and selected controls, we fitted logistic regression models to estimate the effects of serum leptin, insulin, and ghrelin (categorized in tertiles); and because of the skewed distributions of leptin and insulin, we estimated the effects of doubling levels of leptin or insulin by fitting models of $\log_2(\text{leptin})$ or $\log_2(\text{insulin})$, respectively. We fitted similar logistic models to estimate the odds ratio for the effect of diabetes mellitus, insulin, leptin, and ghrelin on BE. Because GERD might mediate the effect of ghrelin on BE, the primary models of ghrelin effects were not adjusted for GERD symptoms or esophagitis. The heterogeneity of the adjusted odds ratios were tested by including in the fully adjusted model terms for GERD and product terms of GERD by diabetes, $\log_2(\text{insulin})$, $\log_2(\text{leptin})$, or ghrelin as a continuous variable.

Results

A total of 1308 male CRC screenees were recruited to undergo upper endoscopy, of which 1202 were eligible, and 851 were enrolled (consent rate 71%). Eight hundred and twenty-two completed the esophagogastroduodenoscopy; 29 were withdrawn from the study due to impact on the clinical throughput of the endoscopy unit ($n = 12$), the inability to adequately sedate for the procedures ($n = 10$), exclusion criteria identified after consent ($n = 4$), or the patient withdrawing consent ($n = 3$).

Compared with the CRC screenees without BE, patients with BE were older, more obese, and more likely to be smokers, have GERD symptoms, and have esophagitis (Table 1). Compared with the cases of BE identified among the CRC screenees, the cases diagnosed by clinically indicated upper endoscopies were more likely to have GERD symptoms, more likely to have tried acid-reducing medications, more likely to have GERD symptoms despite such medications, and less likely to have esophagitis (Table 1).

Among the CRC screenees, those with diabetes mellitus were older, more obese, and were more likely to have smoked and to have GERD symptoms and esophagitis compared with nondiabetic subjects (Supplementary Table 2). Serum insulin was positively associated with obesity and serum leptin and inversely with serum ghrelin (Supplementary Table 3). Serum leptin was positively associated with obesity and inversely associated with current smoking and serum ghrelin (Supplementary Table 4). Serum ghrelin was positively associated with education and income, and inversely associated with obesity and *H pylori* (Supplementary Table 5).

There was little association of diabetes, serum insulin, or serum leptin with GERD, adjusting for potential con-founders (Table 2). Among the nondiabetic patients, serum ghrelin was inversely associated with GERD (adjusted odds ratio [OR] for 3rd vs 1st tertile = 0.364; 95% confidence interval [CI]: 0.195–0.680) (Table 2).

In the total sample, diabetes mellitus was inversely associated with BE, adjusting for potential confounders (OR = 0.680; 95% CI: 0.397–1.17) (Table 3). In contrast, there was a positive association between insulin level and BE among the nondiabetic patients (OR for 3rd vs 1st tertile = 1.67; 95% CI: 0.865–3.21). There was also a positive dose–response association between leptin level and BE (OR for 3rd vs 1st tertile = 3.28; 95% CI: 1.45–7.42). There was a positive association between ghrelin and BE (OR for 400 pg/mL increments of ghrelin = 1.29; 95% CI: 1.03–1.61). We also examined the mutually adjusted effects of insulin, leptin, and ghrelin on BE (Table 3). The estimated effect of insulin was

further attenuated by adjusting for leptin and ghrelin (OR for 3rd vs 1st tertile = 1.14; 95% CI: 0.549–2.38), which was due entirely to the adjustment for leptin and not due to ghrelin (data not shown). However, the estimated effect of leptin was only slightly attenuated by adjusting for insulin and ghrelin (OR for 3rd vs 1st tertile = 3.25; 95% CI: 1.29–8.17), and the estimated effect of ghrelin was slightly strengthened by adjusting for insulin and leptin (OR for 400 pg/mL increments = 1.39; 95% CI: 1.09–1.76). In models that instead treated GERD as a confounder but not a mediator of the ghrelin effect on BE, we found slightly stronger associations between ghrelin and BE (Supplementary Table 6).

When stratifying on GERD status (Table 4), there was a strong inverse association between diabetes mellitus and BE in men with weekly GERD symptoms or erosive esophagitis (OR = 0.383; 95% CI: 0.179–0.821), but not in men without GERD (adjusted OR = 1.26; 95% CI: 0.610–2.58) (*P* for heterogeneity of OR = .02). Insulin was positively associated with BE among those with GERD, but not among those without GERD (Table 4) (*P* for heterogeneity of OR = .08). Leptin was more strongly associated with BE among those with GERD than among those without GERD (Table 4) (*P* for heterogeneity of OR = .01). Among men with GERD, leptin remained strongly associated with BE after further adjustment for insulin and ghrelin (OR for 3rd vs 1st tertile = 5.20; 95% CI: 1.47–18.4). Ghrelin was positively associated with BE among those with GERD (OR for 3rd vs 1st tertile = 2.64; 95% CI: 1.03–6.75), but only weakly associated among those without GERD (*P* for heterogeneity of OR = .85). In analyses restricted to CRC screenees, there were 23 fasting nondiabetic cases of BE with GERD. Among CRC screenees with GERD, there were positive associations with insulin (OR per doubling of insulin = 1.14; 95% CI: 0.822–1.58), leptin (OR per doubling = 1.35; 95% CI: 0.982–1.86), and ghrelin (OR per increment of 400 pg/mL = 1.60; 95% CI: 0.959–2.66), but effect estimates were imprecise.

Discussion

We estimated the effects of diabetes mellitus, serum insulin, leptin, and ghrelin on GERD and BE in men. We found an inverse association of ghrelin with GERD symptoms and erosive esophagitis. Insulin was associated with BE, but its effect appeared to be modified by GERD: hyperinsulinemia was positively associated with BE only among those with GERD. Leptin was also associated with BE, and its effect also appeared to be stronger among men with GERD symptoms. Contrary to our hypothesis, we found a positive association of ghrelin with BE, particularly among men with GERD. The “direct” effect of ghrelin on BE (not mediated by GERD) appears to be positive, and the “indirect” effect mediated through GERD appears to be in the opposite direction (Figure 1). The effect of insulin on BE might be mediated by leptin; the leptin and ghrelin effects did not appear to be confounded by the other measures.

Earlier studies examining the association between insulin levels and BE found only weak associations, but they might have suffered from selection bias of controls, who were typically patients with GERD symptoms undergoing clinical evaluation.^{12–14} In our study, we minimized the potential for selection bias by using CRC screenees as the controls and by using only newly diagnosed cases of BE. Our results suggest that insulin is associated with BE, but only among those with GERD; this effect might be mediated by leptin. We were surprised to find discordant associations of BE with insulin compared with the association with diabetes mellitus. We had hypothesized that diabetics would have had a long period of exposure to hyperinsulinemia and a strong positive association with BE, particularly if we also found a positive association between insulin and BE (Figure 1). Instead, we observed an inverse association between BE and diabetes among men with GERD symptoms. Three earlier studies conflicted regarding the association between diabetes mellitus and esophageal adenocarcinoma.^{15–17} We are unsure of the reason for the discordant associations of BE

with insulin compared with diabetes mellitus in the current study. We can only speculate that it could be due to salutary effects of medications often prescribed to patients with diabetes (eg, aspirin, HMG CoA reductase inhibitors, metformin) or behavioral modifications made by patients after a diagnosis of diabetes, such as dieting and/or exercising.^{34,35} We had insufficient statistical power to explore these possibilities due to the small number of diabetics with GERD symptoms or erosive esophagitis with BE (n = 14). This surprising finding might be due to random error or bias from sparse data.

In 2 earlier studies, circulating leptin was found to be associated with BE, but one did not adjust for abdominal obesity, and the other did not adjust for GERD.^{20,21} We found that the association of leptin with BE among men remained strong when adjusting for abdominal obesity and GERD. In a cell line of esophageal adenocarcinoma, leptin has been shown to stimulate cell proliferation and inhibit apoptosis via cyclooxygenase-2 activation of the epidermal growth factor receptor.¹⁸ In addition, these effects of leptin were synergistic with acid exposure.¹⁹ Although our study was not designed with the intent of analyzing the effects of leptin stratified by GERD, we found stronger estimated effects of leptin on the prevalence of BE in men with GERD than in those without GERD, consistent with earlier work in cell lines. Future studies are needed to elucidate the pathways by which leptin might promote the metaplastic response of BE to GERD. In addition, there might be indirect effects of leptin through alterations in adiponectin, growth hormone, sex hormones, or other adipokines or hormones not measured in this study.

Ghrelin appeared to protect against GERD in our study; perhaps this is mediated by ghrelin promoting gastric emptying (Figure 1).^{14,15} In addition, in a cell line of esophageal adenocarcinoma, ghrelin (500 ng/mL) inhibited the tumor necrosis factor- α induced expressions of cyclooxygenase-2 and interleukin-1 β ,²⁵ so perhaps ghrelin inhibits erosive esophagitis in the setting of GERD. However, to our knowledge, the effects of ghrelin have not been studied at a cellular or tissue level in esophagitis or GERD. In order to explain our findings of a positive association with BE, one could speculate that in individuals with esophagitis, ghrelin might promote healing with a metaplastic response mediated by promotion of growth hormone secretion, including possibly the local production of insulin-like growth factor-1 (Figure 1).^{12,22} Further research is needed to explore these possibilities. We expected to find an inverse association of ghrelin with BE, in part due to the results from an earlier small study (31 cases), which found that very elevated levels of ghrelin (>3200 pg/mL) were inversely associated with the development of esophageal adenocarcinoma, but only among the smaller subset of subjects who were overweight.³⁶ Other studies have found an inverse association between serum ghrelin levels and subsequent development of esophageal squamous cell carcinoma³⁷ and with adenocarcinomas of the stomach and the esophagogastric junction.³⁸ Clearly, additional studies are needed to fully understand the role of ghrelin in the development of BE and esophageal adenocarcinoma.

Our study was limited by the case-control design involving cross-sectional assessments of the 2 outcome conditions and several biological exposures. Therefore, we cannot reliably establish the direction of any causal relations. Our findings might have been biased due to unmeasured confounders, such as other adipokines or the bowel preparations used in our controls, or to misclassified covariates, such as diabetes status, particularly for patients with diet-controlled diabetes. In addition, our study was limited to men, and earlier studies have had conflicting results for the effects of leptin in women on BE.^{20,21} There were differences between our 2 groups of cases of BE related to symptoms of GERD, which we attempted to account for in our analyses by adjusting for GERD severity and esophagitis. There were few cases of BE identified among CRC screenees with GERD, and so it is not possible to simultaneously and completely control for both the potential selection effects and the GERD-specific effects. Our treatment of GERD as an effect modifier was part of an

exploratory analysis that was not planned when we started this effort to estimate the effects of diabetes, insulin, leptin, and ghrelin on BE, therefore, the role of random error (chance) in the analyses stratified by GERD is difficult to assess, and these findings need to be replicated in other populations. Finally, we measured only serum ghrelin and leptin, which might not be as important as ghrelin or leptin present in the gastric lumen that could reflux into the esophagus.

Our study also has several notable strengths. Only patients with newly diagnosed BE were enrolled, reducing the potential effects of volunteer or healthy survivor bias related to patients undergoing endoscopic surveillance. BE required both endoscopic suspicion and histologic confirmation of intestinal metaplasia, which reduced misclassification of our main outcome. The selection of patients screened for CRC was not influenced by the presence of the outcome conditions we were studying, which reduces the risk of selection bias that often threatens the validity of clinical investigations. Finally, we measured a number of important potential confounders and adjusted for those in our analyses.

In conclusion, we found that serum ghrelin is inversely associated with GERD and positively associated with BE. Among men with GERD, we found that elevated serum leptin is positively associated with BE. Hyperinsulinemia was also associated with BE, but this estimated effect might be mediated by leptin. In contrast, we found an inverse association between diabetes mellitus and BE. Confirming these associations and understanding their underlying mechanisms of action could lead to improved control of the rising burden of esophageal adenocarcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

BE	Barrett's esophagus
CI	confidence interval
CRC	colorectal cancer
GERD	gastroesophageal reflux disease
LA	Los Angeles
OR	odds ratio
PPI	proton pump inhibitor

Figure 1.

Potential mechanisms of association of obesity with BE. Obesity mechanically promotes GERD, which can lead to BE. Obesity also leads to insulin resistance and leptin resistance, manifesting as elevated circulating levels of insulin and leptin. Leptin and/or insulin might promote BE, but could also be confounded by the effects of the other. Additionally, the effects of insulin might be mediated through leptin because hyperinsulinemia promotes leptin secretion. We had also hypothesized that diabetes mellitus would be associated with BE due to long-standing hyperinsulinemia. However, in this study, the effect of insulin appeared to be confounded by leptin and ghrelin, and diabetes appeared to be inversely associated with BE. Ghrelin promotes gastric emptying, and so could inhibit GERD. Ghrelin also promotes secretion of growth hormone, which might promote healing of erosive esophagitis with Barrett's metaplasia, either directly, or mediated through local production of insulin-like growth factor-1 (IGF-1).

Table 1
Characteristics of CRC Screenees With and Without BE and Cases of BE Identified by Clinically Indicated Upper Endoscopies

	CRC Screenees		BE identified by clinically indicated
	No BE (n = 751) ^a	BE (n = 70) ^a	EGD (n = 80)
Age, y ^b	58.5 (6.7)	61.0 (6.6)	61.4 (7.1)
Body mass index ^b	29.9 (5.6)	30.3 (4.2)	31.0 (5.6)
Waist-to-hip ratio ^b	1.001 (0.056)	1.020 (0.053)	1.021 (0.054)
Smoking status ^c			
Never	224 (31)	12 (18)	15 (19)
Former	338 (47)	36 (53)	43 (54)
Current	158 (22)	20 (29)	22 (28)
GERD symptoms ^c			
None or less than weekly	604 (83)	46 (66)	15 (19)
without PPI or H2RA			
Weekly or more frequent symptoms	45 (6)	10 (14)	2 (3)
without earlier exposure to PPI or H2RA			
Earlier weekly or more frequent symptoms,	48 (7)	12 (17)	30 (38)
now successfully treated by PPI or H2RA			
Weekly or more frequent symptoms despite taking PPI or H2RA	31 (4)	2 (3)	33 (41)
Esophagitis (LA class B or higher) ^c	75 (10)	18 (26)	3 (4)
Education ^c			
High school or less	150 (21)	17 (26)	24 (30)
Associate degree or some college	265 (37)	27 (41)	34 (43)
Bachelor degree	154 (21)	15 (23)	11 (14)
Doctorate degree	150 (21)	7 (11)	11 (14)
Annual income ^c			
<\$40,000	270 (40)	23 (26)	40 (52)
\$40,000–\$99,999	210 (31)	27 (42)	26 (34)
>\$100,000	197 (29)	14 (22)	11 (14)
Non-Hispanic white ^c	640 (90)	57 (88)	76 (95)
Enrollment site (VA) ^c	325 (43)	34 (49)	44 (55)
Diabetes mellitus ^c	139 (19)	15 (21)	11 (14)

EGD, esophagogastroduodenoscopy; H2RA, histamine-2 receptor antagonist; VA, Ann Arbor Veterans Affairs Medical Center.

^a One CRC screenee was suspected of having BE, but also had esophageal varices, so biopsies were not obtained, and the patient was not classified for BE and instead was excluded from analysis.

^b Data presented as mean (SD).

^c Data presented as number (% without missing data).

Table 2
Estimated Crude and Adjusted Effects of Diabetes Mellitus, Serum Leptin, Serum Insulin, and Serum Ghrelin on GERD

Diabetes/serum insulin/serum leptin/serum ghrelin	No GERD, n/ GERD, n	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
All CRC screenees with GERD or EE, and all CRC screenees without GERD, EE, or BE			
Nondiabetic	460/166	1 (reference)	1 (reference)
Diabetic	91/52	1.58 (1.08–2.33)	1.28 (0.843–1.93) ^b
Fasting nondiabetic subjects: all CRC screenees with GERD or EE, and random sample of CRC screenees without GERD, EE, or BE			
1 st Tertile insulin (<26 pmol/L)	56/45	1 (reference)	1 (reference)
2 nd Tertile insulin (26–48 pmol/L)	53/49	1.15 (0.662–2.00)	1.19 (0.647–2.17)
3 rd Tertile insulin (49–149 pmol/L)	58/47	1.01 (0.582–1.75)	0.895 (0.466–1.72)
Per each doubling of insulin	167/141	0.979 (0.833–1.15)	0.980 (0.817–1.18)
<i>P</i> value for trend		.79	.82
1 st Tertile leptin (<2.4 ng/mL)	60/44	1 (reference)	1 (reference)
2 nd Tertile leptin (2.4–6.7 ng/mL)	55/47	1.17 (0.672–2.02)	0.925 (0.489–1.75)
3 rd Tertile leptin (6.8–20.9 ng/mL)	52/50	1.31 (0.757–2.27)	0.980 (0.477–2.01)
Per each doubling of leptin	167/141	1.01 (0.900–1.13)	0.916 (0.785–1.07)
<i>P</i> value for trend		.87	.26
1 st Tertile ghrelin (<378 pg/mL)	43/59	1 (reference)	1 (reference)
2 nd Tertile ghrelin (378–783 pg/mL)	56/48	0.625 (0.360–1.08)	0.630 (0.349–1.14)
3 rd Tertile ghrelin (784–2700 pg/mL)	68/34	0.364 (0.206–0.644)	0.364 (0.195–0.680)
Per increments of 400 pg/mL ghrelin	167/141	0.731 (0.586–0.913)	0.720 (0.566–0.917)
<i>P</i> value for trend		.006	.008

NOTE. Cases were CRC screenees with at least weekly GERD symptoms before the use of acid-reducing medications or at least LA class B esophagitis. For the analysis of effect of diabetes, controls were all other CRC screenees, and for the analysis of insulin, leptin, and ghrelin, controls were a random sample of nondiabetic CRC screenees who were fasting at least 8 hours at the time of the blood draw.

EE, LA class B erosive esophagitis.

^a Except for the analysis of diabetes mellitus, adjustments were made for age, waist-to-hip ratio (in tertiles), smoking status (never, former, current), *H pylori* serology, insulin, leptin, and ghrelin (each in tertiles).

^b Adjusted for age, waist-to-hip ratio (in tertiles), and smoking status (never, former, current).

Table 3
Estimated Crude and Adjusted Effects of Diabetes Mellitus, Serum Leptin, Serum Insulin, and Serum Ghrelin on Barrett's Esophagus

Diabetes/serum insulin/serum leptin/ serum ghrelin	No BE, n/ BE, n	Crude OR (95% CI)	Adjusted OR ^a (95% CI)	OR (95% CI) further adjusted for insulin, leptin, and ghrelin ^b
Entire cohort				
Nondiabetic	611/124	1 (reference)	1 (reference)	NA
Diabetic	139/26	0.922 (0.581–1.46)	0.680 (0.397–1.17)	NA
Fasting nondiabetic subjects: all subjects with GERD, EE, or BE, and random sample of others				
1 st Tertile insulin (<26 pmol/L)	90/25	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile insulin (26 – 48 pmol/L)	90/25	1.00 (0.534–1.87)	1.06 (0.516–2.17)	0.986 (0.466–2.08)
3 rd Tertile insulin (49 pmol/L)	91/54	2.14 (1.23–3.73)	1.67 (0.865–3.21)	1.14 (0.549–2.38)
Per each doubling of insulin	271/104	1.34 (1.12–1.60)	1.27 (1.04–1.54)	1.15 (0.930–1.42)
<i>P</i> value for trend		.002	.02	.20
1 st Tertile leptin (<2.4 ng/mL)	90/14	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile leptin (2.4–6.7 ng/mL)	90/28	2.00 (0.988–4.05)	1.69 (0.753–3.80)	1.66 (0.710–3.86)
3 rd Tertile leptin (6.8 ng/mL)	91/62	4.38 (2.29–8.38)	3.28 (1.45–7.42)	3.25 (1.29–8.17)
Per each doubling of leptin	271/104	1.44 (1.24–1.67)	1.39 (1.16–1.67)	1.43 (1.16–1.76)
<i>P</i> value for trend		<.001	<.001	<.001
1 st Tertile ghrelin (<378 pg/mL)	90/29	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile ghrelin (378–783 pg/mL)	90/29	1.00 (0.553–1.81)	1.03 (0.544–1.95)	0.903 (0.468–1.74)
3 rd Tertile ghrelin (784 pg/mL)	91/46	1.57 (0.906–2.72)	1.55 (0.828–2.89)	1.59 (0.836–3.02)
Per increments of 400 pg/mL ghrelin	271/104	1.32 (1.08–1.61)	1.29 (1.03–1.61)	1.39 (1.09–1.76)
<i>P</i> value for trend		.006	.03	.007

NOTE. One patient without BE was missing diabetes status, and 1 diabetic CRC screenee was missing BE status (BE was endoscopically suspected, but varices were present and biopsies were not obtained).

EE, LA class B erosive esophagitis; NA, not applicable.

^a Adjusted for age, waist-to-hip ratio (in tertiles), smoking status (never, former, current), *H pylori* serology, esophagitis (LA class B or higher esophagitis vs none or LA class A), and GERD (4 categories as described in Methods), except analysis of diabetes effect, which was not adjusted for *H pylori* serology, and analysis of ghrelin effect was not adjusted for esophagitis or GERD.

^b Adjusted for the same variables as above, plus insulin, leptin, and ghrelin (each in tertiles).

Table 4
Estimated Crude and Adjusted Effects of Diabetes Mellitus, Serum Insulin, Serum Ghrelin, and Serum Ghrelin on BE by GERD

	Less than weekly GERD symptoms and less than LA class B esophagitis				Weekly or more frequent GERD symptoms or LA class B or higher esophagitis			
	No BE, n/BE, n	Crude OR (95% CI)	Adjusted OR ^a (95% CI)	OR (95% CI) further adjusted for insulin, leptin, and ghrelin ^b	No BE, n/BE, n	Crude OR (95% CI)	Adjusted OR ^c (95% CI)	OR (95% CI) further adjusted for insulin, leptin, and ghrelin ^d
Diabetes/serum insulin/serum leptin								
Entire cohort								
Nondiabetic	460/39	1 (reference)	1 (reference)	NA	139 / 85	1 (reference)	1 (reference)	NA
Diabetic	91/12	1.56 (0.784–3.09)	1.26 (0.610–2.58)	NA	45 / 14	0.509 (0.264–0.982)	0.383 (0.179–0.821)	NA
Fasting nondiabetic subjects: all subjects with GERD, EE, or BE and random sample of others								
1 st Tertile insulin (<3.8 mU/L)	51/11	1 (reference)	1 (reference)	1 (reference)	38 / 14	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile insulin (3.8–6.9 mU/L)	45/9	0.927 (0.352–2.44)	1.10 (0.374–3.26)	1.19 (0.379–3.71)	44 / 16	0.987 (0.427–2.28)	1.05 (0.404–2.72)	0.922 (0.332–2.56)
3 rd Tertile insulin (7.0 mU/L)	52/11	0.981 (0.391–2.46)	0.771 (0.266–2.23)	0.569 (0.168–1.92)	36 / 43	3.24 (1.52–6.91)	2.59 (1.09–6.12)	1.58 (0.606–4.11)
Per each doubling of insulin	148/31	1.12 (0.815–1.54)	1.08 (0.740–1.57)	1.01 (0.667–1.54)	118 / 73	1.41 (1.13–1.76)	1.35 (1.06–1.71)	1.19 (0.926–1.53)
<i>P</i> value for trend		.49	.70	.95		.003	.01	.17
1 st Tertile leptin (<2.4 ng/mL)	51/7	1 (reference)	1 (reference)	1 (reference)	39 / 7	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile leptin (2.4–6.7 ng/mL)	49/9	1.34 (0.462–3.87)	0.703 (0.191–2.60)	0.771 (0.191–3.12)	39 / 19	2.71 (1.03–7.19)	2.83 (0.944–8.49)	2.27 (0.715–7.21)
3 rd Tertile leptin (6.8 ng/mL)	48/15	2.28 (0.855–6.07)	1.39 (0.393–4.89)	1.97 (0.459–8.50)	40 / 47	6.55 (2.64–16.2)	6.50 (2.11–20.0)	5.20 (1.47–18.4)
Per each doubling of leptin	148/31	1.17 (0.941–1.46)	1.09 (0.821–1.44)	1.14 (0.833–1.56)	118 / 73	1.65 (1.33–2.04)	1.68 (1.30–2.17)	1.71 (1.26–2.31)
<i>P</i> value for trend		0.16	0.56	0.41		< 0.001	< 0.001	< 0.001
1 st Tertile ghrelin (<378 pg/mL)	38/7	1 (reference)	1 (reference)	1 (reference)	51 / 22	1 (reference)	1 (reference)	1 (reference)
2 nd Tertile ghrelin (378–783 pg/mL)	47/8	0.924 (0.307–2.78)	1.18 (0.343–4.06)	1.03 (0.291–3.62)	42 / 21	1.09 (0.821–1.44)	0.938 (0.404–2.18)	0.967 (0.399–2.35)
3 rd Tertile ghrelin (784 pg/mL)	63/16	1.38 (0.520–3.66)	1.27 (0.402–4.02)	1.15 (0.359–3.68)	25 / 30	2.78 (1.34–5.77)	2.26 (0.936–5.47)	2.64 (1.03–6.75)
Per increments of 400 pg/mL Ghrelin	148/31	1.36 (0.982–1.89)	1.25 (0.856–1.82)	1.25 (0.838–1.86)	118 / 73	1.52 (1.15–2.00)	1.44 (1.03–2.01)	1.67 (1.16–2.41)
<i>P</i> value for trend		.06	.25	.28		.003	.03	.006

NOTE. Fifteen controls without BE presented in Table 2 (5 of whom had been randomly selected for assaying of insulin and leptin) were missing data on GERD frequency when not taking PPIs or H2RAs and did not have esophagitis, and so were excluded from this stratified analysis. EE, LA class B erosive esophagitis; NA, not applicable.

^a Adjusted for age, waist-to-hip ratio (in tertiles), *H pylori* serology, and smoking status (current, former, or never), except analysis of diabetes effect, which was not adjusted for *H pylori* serology.

- ^b Adjusted for leptin, insulin, and ghrelin (in tertiles), age, waist-to-hip ratio (in tertiles), *H pylori* serology, and smoking status (current, former, or never).
- ^c Adjusted for age, waist-to-hip ratio (in tertiles), smoking status (current, former, or never), *H pylori* serology, GERD symptom response to PPIs or H2RAs (no prior exposure, less than weekly symptoms when using such medications, or persistent weekly symptoms despite such medications), and esophagitis (LA class B or higher esophagitis vs none or LA class A), except analysis of diabetes effect, which was not adjusted for *H pylori* serology.
- ^d Adjusted for leptin, insulin, and ghrelin (in tertiles), age, waist-to-hip ratio (in tertiles), smoking status (current, former, or never), *H pylori* serology, GERD symptom response to PPIs or H2RAs (no prior exposure, less than weekly symptoms when using such medications, or persistent weekly symptoms despite such medications), and esophagitis (LA class B or higher esophagitis vs none or LA class A).