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

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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 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 FOXF1, 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

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.

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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, eosophagitis, 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 $\log_2$(leptin) or $\log_2$(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, $\log_2$(insulin), $\log_2$(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,