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ORIGINAL ARTICLE

Ghrelin and Leptin Have a Complex Relationship with Risk of Barrett's Esophagus

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Abstract
Background Abdominal obesity is a risk factor for Barrett's esophagus independent of GERD symptoms, but little is understood about the biological mechanisms between obesity and the carcinogenic pathway of esophageal adenocarcinoma.
Aims To evaluate whether ghrelin and leptin may partially explain the association between obesity and Barrett's esophagus.
Methods We conducted a case–control study using patients with a new diagnosis of Barrett's esophagus (cases) and two control groups frequency matched to cases for age, gender, and geographic region: (1) patients with gastroesophageal reflux disease (GERD) and (2) a sample of the general population. We generated odds ratios using logistic regressions to evaluate quartiles of serum ghrelin or serum leptin, adjusting for known risk factors for Barrett's esophagus. We evaluated potential interaction variables using cross products and ran stratified analyses to generate stratum-specific odds ratios.
Results A total of 886 participants were included in the analysis. Higher ghrelin concentrations were associated with an increased risk of Barrett's esophagus, when compared to the population controls, but not the GERD controls. Ghrelin concentrations were not associated with the frequency of GERD symptoms, but ghrelin's relationship with Barrett's esophagus varied significantly with the frequency of GERD symptoms. Leptin concentrations were positively associated with at least weekly GERD symptoms among the population controls and were inversely associated with Barrett's esophagus only among the GERD controls. Adjusting for waist circumference did not change the main associations.
Conclusion Higher levels of ghrelin were associated with an increased risk of Barrett's esophagus among the general population. In contrast, leptin was positively associated with frequent GERD symptoms, but inversely associated with the risk of Barrett's esophagus among the GERD controls.

Keywords Barrett's esophagus · GERD · Obesity · Ghrelin · Leptin

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Introduction

The incidence of esophageal adenocarcinoma has increased dramatically over the past half century and continues to increase, though at a slower pace [1–4]. There are several

risk factors for esophageal adenocarcinoma, including older age, Caucasian race, male gender, smoking tobacco, abdominal obesity, and gastroesophageal reflux disease (GERD) [5–8]. Perhaps the most important risk factor for esophageal adenocarcinoma is the presence of Barrett's esophagus on endoscopy.

Barrett's esophagus is a precursor lesion for esophageal adenocarcinoma and is associated with an increased risk of esophageal adenocarcinoma by as much as 60-fold [9]. It is metaplasia of the lower esophagus, likely due to an aberrant healing process induced by esophageal injury, typically from GERD. While it is not clear why some patients develop Barrett's esophagus and others do not, risk factors have been identified, such as GERD, age, gender, socioeconomic status, smoking tobacco, and abdominal obesity [5, 7, 10–15]; many of these overlap the risk factors for esophageal adenocarcinoma.

The association between abdominal obesity and Barrett's esophagus could potentially explain the increasing incidence of esophageal adenocarcinoma [16]. Abdominal obesity is associated with GERD, a known risk factor for Barrett's esophagus. Abdominal obesity is also associated with Barrett's esophagus, but this relationship appears to be independent of reflux symptoms [11]. Several endogenous compounds, including ghrelin and leptin, are associated with obesity and may modify gastrointestinal response to injury or function, which potentially could partially explain the links between abdominal obesity and Barrett's esophagus.

Ghrelin is a peptide hormone produced by the gastric fundus and upper gastrointestinal tract, which stimulates appetite, promotes gastric motility, and modifies inflammatory pathways [17–22]. Low levels of serum ghrelin have been found to be associated with obesity, GERD, and *Helicobacter Pylori* (*H. Pylori*) infection [22–25]. High levels of ghrelin are inversely associated with esophageal adenocarcinoma [26, 27]. However, a recent study investigated the relationship between ghrelin and Barrett's esophagus and found a positive association with high levels of ghrelin [28], which is difficult to reconcile with the reported relationships between ghrelin and some of the known risk factors for Barrett's esophagus.

Leptin is a hormone produced by adipocytes that has a key role in the regulation of energy balance and a large number of other important physiological processes and is elevated in patients with a higher body mass index (BMI), increased body adiposity, and larger waist circumference [23, 29]. Leptin is a pro-inflammatory peptide [30] and stimulates growth and inhibits apoptosis in esophageal adenocarcinoma cell lines [31, 32]. Patients with an *H. Pylori* infection have lower circulating leptin concentrations [24], providing, in addition to the relationship between *H. Pylori* and gastroesophageal reflux [33], an additional mechanism through which *H. Pylori* infection may be protective of Barrett's esophagus. Such evidence suggests leptin may play a role in mediating the pathogenesis of Barrett's esophagus.

The aim of this study was to investigate the possible relationships between Barrett's esophagus and the endogenous peptides, ghrelin, and leptin (Fig. 1), at the time of diagnosis of Barrett's esophagus using a case–control study of men and women with population and GERD control groups, and whether they potentially have a role as biological mediators of Barrett's esophagus with known epidemiological risk factors.

Methods

Study Population

The study population was drawn from among 3 million members of Kaiser Permanente Northern California (KPNC), an integrated healthcare delivery organization. The KPNC population closely approximates that of the general population in the Northern California region [34]. Those selected were between ages 18 and 79, had been members for at least 2 years prior to their index date, met selection criteria described below, and were able to understand written and spoken English. The KPNC Institutional Review Board approved the analyses and the study design.

Case Definition

Potential cases included all KPNC members with a new diagnosis of Barrett's esophagus (to minimize selection bias) between October 2002 and September 2005. The index date for these cases was the date of diagnosis with Barrett's esophagus. These patients were identified using ICD-9 code 530.2, which was recorded as Barrett's esophagus in KPNC. A board-certified gastroenterologist

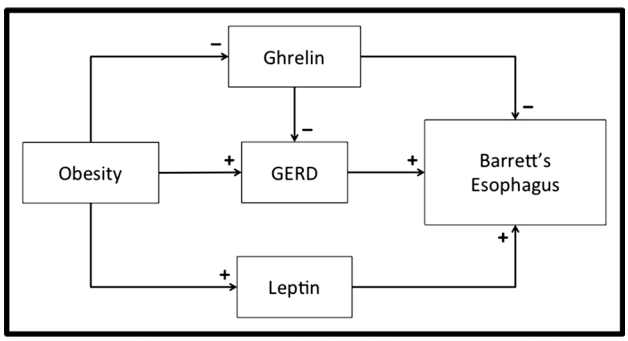

Fig. 1 Possible relationships between obesity, ghrelin, leptin, gastroesophageal reflux disease (GERD), and Barrett's esophagus

(DAC) reviewed the endoscopy and pathology reports for the potential cases. Cases with findings consistent with recommended definitions of Barrett's esophagus were included: an EGD report describing a visible length of columnar-type epithelium proximal to the gastroesophageal junction/gastric folds; a biopsy of this area was performed, and the biopsy showed intestinal-type columnar epithelium [35]. A gastrointestinal pathologist conducted a separate manual review of the pathology slides. Patients were not included if they had any of the following: only gastric-type or columnar-type metaplasia (without intestinal metaplasia) of the esophagus on all pathologic evaluations; no biopsy specimens of esophageal origin; biopsy specimens of only a mildly irregular squamocolumnar junction (i.e., irregular *z*-line); or a prior diagnosis of Barrett's esophagus.

Control Groups

Two control groups were used for this study. The first was a GERD control group of KPNC members who had all of the following characteristics: a GERD-related ICD-9 code (530.11 [reflux esophagitis] or 530.81 [gastroesophageal reflux]); a prescription sufficient for at least 90 days use of H2RB or a PPI within the year previous to the index date; no prior diagnosis of Barrett's esophagus in electronic coding; and an esophagogastroduodenoscopy (EGD) performed in close proximity to the index date that did not show esophageal columnar metaplasia of any type. A board-certified gastroenterologist (DAC) reviewed the EGD and pathology reports.

The second control group, the population control group, was randomly selected from the general population within KPNC membership using risk set sampling (this is all people at any given point in time who are theoretically at risk of developing Barrett's esophagus). These patients had no prior diagnosis of GERD or Barrett's esophagus before the index date. The index dates for both population and GERD controls were determined to be the midpoint of a 2- to 3-month selection interval for the cases. The GERD and population controls were frequency matched to cases by gender, age at the index date (by 5-year age groups), and geographic region (each subject's home medical facility).

Data Collection

All subjects completed an in-person interview related to GERD symptoms and frequency, medical history, and use of medications, tobacco, and alcohol; a validated food frequency questionnaire (the Block 1998 full-length, 110 food items) [36–39]; and measurement of height, body weight, and waist/thigh circumferences. Participants were asked to report exposures for the year prior to the index date. Trained interviewers completed these examinations, most commonly at the subject's home, using standardized equipment. GERD symptoms were assessed using a validated symptom questionnaire and were defined as the presence of either heartburn (a burning pain or discomfort behind the breastbone) or acid regurgitation (a bitter- or sour-tasting fluid coming up into the throat or mouth) [40].

Serum Measurements

Serum samples were collected from study subjects at the time of examination and stored at −80 °C for laboratory analyses, which were performed in 2011. Plasma leptin was measured in duplicate using a radioimmunoassay (EMD, Millipore, St. Charles, MO) that utilized human antiserum and 125I-peptides. The sensitivity (lower limit of detection) for the assay is 0.44 ng/mL. The mean intra- and inter-assay coefficients of variation for the leptin assay in Dr. Havel's laboratory were 6.6 and 8.4 %, respectively. Plasma ghrelin was measured in duplicate by radioimmunoassay (Phoenix Pharmaceuticals, Inc., Belmont, CA) using a rabbit antiserum and 125I-ghrelin as a tracer. The sensitivity (lower limit of detection) of the assay was 45 pg/mL. The mean intra- and inter-assay coefficients of variation for the ghrelin assay in Dr. Havel's laboratory were 4.3 and 12.3 %, respectively.

Statistical Analysis

We employed standard analytic techniques for an unpaired case–control study, including unconditional logistic regression [41–43]. Comparisons of proportions used the binomial distribution. Analyses were performed using STATA version 12.1 (STATA Corp, College Station, TX, USA). The odds ratio was considered an estimate of relative risk, given the low prevalence of Barrett's esophagus [44]. Quartiles were generated for serum leptin and serum ghrelin, using the distributions among the population controls to define those quartiles. Trend analyses utilized the *p* value across the categorical quartile variable. All analyses utilizing serum leptin or serum ghrelin as a continuous variable were log-transformed to normalize the distribution.

Based on a review of the literature, we included the following potential confounders in the models: age, gender, race, smoking status (never smoked, smoked in the past, currently smoking), alcohol use (ever vs. never used), and *H. Pylori* serology (serum antibody positive or negative). In addition, the following variables were evaluated as potential confounders and kept if inclusion in the model changed the adjusted main effect odds ratio by 10 % or more: education (<7, 7–9, 10–11, >11 years), waist circumference, and a comorbidity index incorporating

demographic data, medical coding, and pharmacy utilization. Using this method, we identified waist circumference as a potential confounder.

We evaluated each of the following variables for the presence of effect modification: race, *H. Pylori* serology, frequency of GERD symptoms (no GERD symptoms or occurring less once per week vs. at least weekly symptoms), BMI, and smoking status. We tested each variable against each of the peptides in our study (ghrelin and leptin) and against both control groups (GERD and population). This was performed using a logistic regression that included the outcome (cases of Barrett's esophagus vs. control group), the log-adjusted serum peptide levels, the potential effect modifier, and an interaction term (the product of the log-adjusted serum adipokine and the potential effect modifier). Stratum-specific odds ratios were then evaluated for each variable that had a statistically significant interaction term ($p < 0.05$).

Supplementary analyses were performed after completing the main analyses. The first of these evaluated the effect of including waist circumference or *H. Pylori* serology in the main analysis had on the odds ratio for ghrelin and leptin. We also evaluated ghrelin and leptin for a relationship with GERD symptoms occurring at least weekly. We performed unconditional logistic regressions to generate an odds ratio using quartiles of ghrelin and leptin and frequency of GERD symptoms. The models were adjusted for waist circumference.

Results

Patient Characteristics

A total of 953 patients were initially recruited into the study; these included 320 cases of Barrett's esophagus, 316 GERD controls, and 317 population controls. Of them, 886 patients had complete questionnaire and laboratory data for all relevant variables and were included in the analysis. The remaining 67 were excluded for missing or invalid data for the following variables: serum leptin and ghrelin ($n = 24$), waist circumference ($n = 1$), weight ($n = 2$), race/ethnicity ($n = 5$), smoking status ($n = 1$), GERD score ($n = 1$), and *H. Pylori* status ($n = 33$). The characteristics of each group are summarized in Table 1. Patients in the population controls were less likely to have at least weekly GERD symptoms (27 %) compared to the GERD controls (75 %) or cases of Barrett's esophagus (80 %).

Ghrelin

Higher serum concentrations of ghrelin were positively associated with an increased risk of Barrett's esophagus, when compared to the population controls (fourth quartile vs. first quartile, adjusted odds ratio [OR] 1.87, 95 % confidence interval [95 % CI] 1.11–3.14), with a significant test for trend across quartiles ($p = 0.05$) (Table 2), although most of the increased risk occurred between the first and second quartiles. However, no statistically significant associations between ghrelin and Barrett's esophagus were seen in the comparisons with the GERD controls (fourth quartile vs. first quartile, OR 1.20, 95 % CI 0.73–1.98) (Table 2).

Among the population controls, the association between ghrelin concentrations and Barrett's esophagus varied with the frequency of GERD symptoms (p interaction $= 0.024$), with substantial differences in risk across the quartiles between patients with versus without at least weekly GERD symptoms (Table 3). The fourth quartile of ghrelin was associated with an increased risk of Barrett's esophagus among those with at least weekly GERD symptoms (fourth vs. first quartile OR 2.48, 95 % CI 1.08–5.68), but not among those with less than weekly or no GERD symptoms (fourth vs. first quartile OR 0.99, 95 % CI 0.30–3.22) (Table 3). In contrast, the second and third quartiles of ghrelin were associated with Barrett's esophagus risk only among those with less than weekly or no GERD symptoms (Table 3). When compared to the GERD controls, the associations between ghrelin and Barrett's esophagus were similar for patients with versus without at least weekly GERD symptoms (p interaction $= 0.17$) although there were few patients among the case or GERD controls without GERD symptoms, limiting our ability to evaluate for interaction between these populations. We did not observe any other interactions between ghrelin and the other variables we tested for effect modification.

In a supplementary analysis evaluating the relationship between ghrelin and GERD symptom frequency, we did not find a significant relationship between ghrelin and GERD symptoms occurring at least weekly when adjusting for waist circumference (fourth quartile vs. first quartile OR 1.11, 95 % CI 0.49–2.53) or when not adjusting for waist circumference in the model (fourth quartile vs. first quartile OR 0.70, 95 % CI 0.32–1.50).

Leptin

We found no association between levels of serum leptin concentrations and Barrett's esophagus for comparisons with all the population controls (fourth vs. first quartile OR 1.49, 95 % CI 0.73–3.05); but there were significant inverse associations among GERD controls (fourth vs. first quartile OR 0.50, 95 % CI 0.24–1.01; p values for trend 0.04) (Table 2). We did not observe any interactions between leptin and GERD symptoms occurring at least weekly with the population controls (p interaction $= 0.17$) or the GERD controls (p interaction $= 0.24$). Neither did we observe any significant interactions between leptin and

Table 1 Patient characteristics

	Cases of BE	GERD controls	Population controls
No. of subjects, *n*	300 (100 %)	296 (100 %)	290 (100 %)
Age in years, mean (SD)	62 (11)	62 (11)	62 (10)
Age, *n* (%)			
20–39	7 (2 %)	10 (3 %)	9 (3 %)
40–59	115 (38 %)	108 (37 %)	98 (34 %)
60–79	178 (60 %)	178 (60 %)	183 (63 %)
Race, *n* (%)			
Non-Hispanic white	263 (88 %)	240 (81 %)	247 (85 %)
Black	2 (1 %)	16 (5 %)	15 (5 %)
Hispanic	24 (8 %)	20 (7 %)	11 (4 %)
Asian	3 (1 %)	7 (2 %)	8 (3 %)
Other	8 (3 %)	13 (4 %)	9 (3 %)
Sex, *n* (%)			
Male	218 (73 %)	204 (69 %)	198 (68 %)
Female	82 (27 %)	92 (31 %)	92 (32 %)
GERD symptom frequency, *n* (%)			
<l/week	60 (20 %)	75 (25 %)	211 (73 %)
>or =1/week	240 (80 %)	221 (75 %)	79 (27 %)
Waist circumference in cm, mean (SD)	100.4 (14.5)	97.1 (14.3)	99.1 (16.6)
Weight in lbs, mean (SD)	190.6 (45.8)	185.2 (35.6)	192.5 (40.5)
BMI, *n* (%)			
<19	4 (1 %)	2 (1 %)	1 (<1 %)
19–25	58 (19 %)	61 (21 %)	64 (22 %)
26–30	121 (41 %)	131 (44 %)	109 (38 %)
>30	117 (39 %)	102 (34 %)	116 (40 %)
Smoking status, *n* (%)			
Currently smoking	39 (13 %)	29 (10 %)	31 (11 %)
Formerly smoked	161 (54 %)	145 (49 %)	127 (44 %)
Never smoked	100 (33 %)	122 (41 %)	132 (45 %)
Helicobacter pylori serology, *n* (%)			
Negative	265 (88 %)	267 (90 %)	223 (77 %)
Positive	35 (12 %)	29 (10 %)	67 (23 %)
Serum ghrelin in pg/mL, mean (SD)	346.0 (209.4)	336.4 (194.9)	310.0 (165.8)
Quartile, mean (SD)			
First quartile (<191.9 pg/mL)	158.1 (25.3)	151.3 (29.1)	154.3 (24.9)
Second quartile (191.9–269.2 pg/mL)	227.8 (22.6)	230.4 (22.6)	230.1 (21.5)
Third quartile (269.3–371.4 pg/mL)	321.2 (29.1)	317.4 (29.4)	315.1 (30.0)
Fourth quartile (>371.4 pg/mL)	569.4 (239.0)	549.7 (199.5)	542.8 (153.1)
Serum leptin in ng/mL, mean (SD)	16.15 (16.55)	16.66 (15.44)	15.62 (15.21)
Quartile, mean (SD)			
First quartile (<5.97 ng/mL	4.21 (1.22)	3.67 (1.22)	4.20 (1.06)
Second quartile (5.97–10.21 ng/mL)	7.76 (1.29)	7.76 (1.14)	8.00 (1.28)
Third quartile (10.22–19.655 ng/mL)	14.46 (2.80)	13.92 (2.75)	14.01 (2.76)
Fourth quartile (>19.655 ng/mL)	36.54 (21.09)	37.87 (15.20)	36.45 (17.20)

BMI categories arc based on international standards as presented by the WHO Global Database on BMI

gender with the population controls (p interaction = 0.83) or the GERD controls (p interaction = 0.19). No other variables were found to interact with leptin and Barrett's esophagus in our evaluation of possible effect modification.

In a supplementary analysis evaluating the relationship between leptin and GERD symptom frequency, we found leptin was positively associated with GERD symptom frequency among the population controls (fourth vs. first

Table 2 Ghrelin, leptin, and cases of Barrett's esophagus

	No. of case/control	Crude OR (95 % CI)	Partially adjusted[a] OR (95 % CI)	Fully adjusted[b] OR (95 % CI)
Ghrelin				
Cases of BE versus population controls				
First quartile	51/73	Ref	Ref	Ref
Second quartile	81/72	1.61 (0.99. 2.60)	1.54 (0.94. 2.52)	1.57 (0.96, 2.58)
Third quartile	74/73	1.45 (0.90, 2 35)	1.25 (0.76. 2.07)	1.29 (0.78, 2.14)
Fourth quartile	94/72	1.87 (1.17, 2.99)	1.77 (1.07, 2.92)	1.87 (1.11, 3.14)
p value for trend		0.02	0.07	0.05
Cases of BE versus GERD controls				
First quartile	51/58	Ref	Ref	Ref
Second quartile	81/81	1.14 (0.70, 1.85)	1.16 (0.71, 1.90)	1.21 (0.74, 2.00)
Third quartile	74/61	1.38 (0.83, 2.29)	1.44 (0.86, 2.41)	1.55 (0.92, 2.62)
Fourth quartile	94/96	1.11 (0.69, 1.78)	1.05 (0.65, 1.72)	1.20 (0.73. 1.98
p value for trend		0.62	0.81	0.44
Leptin				
Cases of BE versus population controls				
First quartile	70/73	Ref	Ref	Ref
Second quartile	71/72	1.03 (0.65, 1.63)	0.99 (0.62, 1.60)	1.04 (0.63, 1.69)
Third quartile	82/73	1.17 (0.74, 1.85)	1.34 (0.83, 2.18)	1.47 (0.84, 2.56)
Fourth quartile	77/72	1.12 (0.70, 1.77)	1.27 (0.74, 2.19)	1.49 (0.73, 3.05)
p value for trend		0.54	0.24	0.18
Cases of BE versus GERD controls				
First quartile	70/63	Ref	Ref	Ref
Second quartile	71/67	0.95 (0.59, 1.54)	0.98 (0.60, 1.59)	0.79 (0.47, 1.30)
Third quartile	82/88	0.84 (0.53, 1.32)	0.91 (0.56, 1.46)	0.60 (0.35, 1.04)
Fourth quartile	77/78	0.89 (0.56, 1.41)	1.01 (0.59, 1.74)	0.50 (0.24, 1.01)
p value for trend		0.52	0.93	0.04

[a] Adjusted for smoking status, alcohol use, *H. Pylori* serology, age, sex, and race

[b] Adjusted for waist circumference, smoking status, alcohol use, *H. Pylori* serology, age, sex, and race

Table 3 Ghrelin and cases of Barrett's esophagus by GERD symptom frequency

	Symptoms < 1/week		Symptoms > or =1/week	
	No. of case/control	OR (95 % CI)	No. of case/control	OR (95 % CI)
Ghrelin				
Cases of BE versus. population controls				
First quartile	8/53	Ref	43/20	Ref
Second quartile	21/51	3.30 (1.25, 8.68)	60/21	1.30 (0.61, 2.76)
Third quartile	24/50	3.41 (1.27, 9.15)	50/23	0.96 (0.45, 2.06)
Fourth quartile	7/57	0.99 (0.30, 3.22)	87/15	2.48 (1.08, 5.68)

Adjusted for waist circumference, smoking status, alcohol use, *H. Pylori* serology, age, sex, and race

quartile OR 3.81, 95 % CI 1.57–9.25). This relationship was attenuated when we adjusted for waist circumference (fourth vs. first quartile OR 2.51, 95 % CI 0.97–6.46).

Supplementary Analyses

Abdominal obesity and *H. pylori* are both associated with Barrett's esophagus and with some adipokines [5, 11, 15, 45–47]. Given these known associations, we evaluated whether the individual inclusion/exclusion of H. pylori infection and waist circumference influenced the associations found; if present, this which would suggest that ghrelin and leptin may be in the biological pathway between these factors and the risk of Barrett's esophagus.

There was no strong evidence that ghrelin or leptin were in the same biological pathway as *H. pylori* or waist

circumference and Barrett's esophagus. For ghrelin, among the population controls, the inclusion of waist circumference in the model changed the odds ratio of the fourth quartile versus the first quartile between ghrelin and Barrett's esophagus from 1.77 (95 % CI 1.07–2.92) to 1.87 (95 % CI 1.11–3.14) in a model containing other potential confounders (age, sex, smoking, race, alcohol, and H. pylori). For leptin, the inclusion of waist circumference increased the odds ratio of the fourth quartile versus the first quartile from 1.27 (95 % CI 0.74–2.19) to 1.49 (95 % CI 0.73–3.05).

For ghrelin, among the population controls, the inclusion of *H. pylori* in the model changed the odds ratio of the fourth quartile versus the first quartile from 2.07 (95 % CI 1.24–3.44) to 1.87 (95 % CI 1.11–3.14) in a model containing other potential confounders (age, sex, smoking, race, alcohol, and waist circumference). For leptin, the inclusion of *H. pylori* changed the odds ratio of the fourth quartile versus the first quartile from 1.65 (95 % CI 0.81–3.37) to 1.49 (95 % CI 0.73–3.05).

Given the presence of associations with the population controls, but not with the GERD controls, we evaluated ghrelin and leptin for a possible relationship with GERD. We did not find a significant relationship between ghrelin and GERD symptoms occurring at least weekly (fourth quartile vs. first quartile OR 0.70, 95 % CI 0.32–1.50), neither was a relationship seen when adjusted for waist circumference (fourth quartile vs. first quartile OR 1.11, 95 % CI 0.49–2.53). We found leptin was positively associated with GERD symptom frequency among the population controls (fourth vs. first quartile OR 3.81, 95 % CI 1.57–9.25). When adjusted for waist circumference, this relationship was attenuated (fourth vs. first quartile OR 2.51, 95 % CI 0.97–6.46).

Discussion

The principal goal of this study was to evaluate the relationships between ghrelin, leptin, and Barrett's esophagus. We did this by utilizing both population and GERD controls and found that higher ghrelin levels had a positive association with the risk of Barrett's esophagus relative to the population controls, but not the GERD controls. We did not find a relationship between ghrelin and GERD in our supplementary analysis, suggesting ghrelin's relationship with Barrett's esophagus is independent of GERD. We evaluated several variables for potential effect modification and found GERD interacted with ghrelin in its relationship with Barrett's esophagus, among the highest levels of ghrelin, though this relationship may be misleading as this analysis was limited by the small sample size of some of the strata. Interpretation is further limited by the inconsistent results for the lower ghrelin levels. An inverse association (by trend analysis) was found between leptin and Barrett's esophagus, but only among comparisons with the GERD controls.

Our findings with ghrelin are contrary to what we expected from reviewing the literature. We hypothesized that ghrelin would have an inverse relationship with Barrett's esophagus, similar to that described in the relationship between ghrelin and esophageal adenocarcinoma [26, 27]. However, Rubenstein et al. also reported a positive association between ghrelin and Barrett's esophagus [28] and also demonstrated that this association was seen among those with more frequent GERD symptoms [28].

We also aimed to determine whether the relationship between circulating ghrelin levels and Barrett's esophagus might be influenced by GERD, as it is a risk factor for Barrett's esophagus [8, 15]. Given that some of the highest levels of ghrelin were associated with Barrett's esophagus among those with more frequent symptoms in the stratified analysis, we would have expected the same relationship among the GERD controls, which reported more frequent GERD symptoms than the population controls (Table 1). Instead, we found there was no relationship when compared to the GERD controls. One possibility is that ghrelin's effect on Barrett's esophagus is through its effect on GERD, in which case we would expect a positive relationship between ghrelin and GERD. However, we found no association between ghrelin and GERD, and Rubenstein et al. found an inverse association [28]. Another possibility for this inconsistency was proposed by Rubenstein et al. that ghrelin had both a positive relationship and an inverse relationship with Barrett's esophagus, whereby ghrelin may be associated with a reduced risk of GERD itself, but an increased risk of abnormal healing of mucosal injury into esophageal intestinal metaplasia (i.e., Barrett's esophagus) [28]. This might explain why we did not detect a relationship among our GERD control group as they had a higher frequency of GERD symptoms than the population controls, and the protective benefits of ghrelin may have attenuated any relationship between ghrelin and Barrett's esophagus not mediated by GERD, but not the absence of a relationship between ghrelin and GERD.

The present study, which was well powered and included both male and female participants as well as population and GERD controls, did not demonstrate any relationship between leptin and Barrett's esophagus among the population controls, but an inverse association was seen among the GERD controls, despite finding that leptin is positively associated with GERD symptoms among the population controls. This observation differs somewhat from some studies to date [28, 48–50]. Rubenstein et al. examined the relationship of leptin and Barrett's esophagus in a male-only population and found a positive relationship (third

quartile vs. first quartile OR 3.25, 95 % CI 1.29–8.17) [28]. Garcia et al. also found a positive association between leptin and Barrett's esophagus (fifth quartile vs. first quartile OR 8.02, 95 % CI 2.79–23.07), but they neither controlled nor adjusted for GERD in their study design [48]. The marked difference in our findings from these studies could be attributed to the differences in study population as both used a male-only population and used cases of Barrett's esophagus diagnosed as part of a study protocol, although 80 out of 150 of the patients with Barrett's esophagus in Rubenstein et al. were diagnosed on a clinically indicated esophagogastroduodenoscopy [28, 48]. We tested gender for effect modification but did not find a significant interaction. While our study included more females than other studies, it may have been underpowered in the test for effect modification by gender. However, other studies have conflicting data on how gender affects the relationship between leptin and Barrett's esophagus [49, 50]. One study saw a positive relationship among its female participants but not among the male participants nor when the two genders were combined [49]. In contrast, another study saw a positive association among its male patients and an inverse association among its female patients [50]. Given these inconsistent findings, it seems likely that unmeasured variables that differ between patient populations or, alternatively, differences in the collection or assay methods for leptin, may be present in the different studies of the relationship between leptin and Barrett's esophagus.

Finally, our study did not find that inclusion in the model of abdominal obesity (as assessed by waist circumference) and, separately, *H. pylori*, influenced the associations between ghrelin, leptin, and Barrett's esophagus. This suggests that abdominal obesity and *H. pylori* influence Barrett's esophagus independently of circulating ghrelin and leptin concentrations.

This study has several strengths. First, our cases of Barrett's esophagus were recruited close to the time of diagnosis, minimizing selection bias that may result from patients modifying their behaviors after the diagnosis. Second, we had population and GERD control groups, the latter with a negative endoscopy for Barrett's esophagus, allowing us to examine the potential interactions explaining why Barrett's esophagus occurs in some patients with GERD and not others. Third, study participants were recruited from a population that closely parallels the general population, making these findings easier to generalize and less subject to selection bias. Fourth, we examined several variables for possible interactions, which could explain conflicting results seen in previous studies.

There are also several limitations to this study. Case–control studies cannot establish causality, limiting the conclusions that can be drawn from the results. The presence of incomplete control of confounding among the measured confounders, or the presence of unmeasured confounders, may influence the results. Lastly, while adequately powered for the main analysis, we were not adequately powered to evaluate potential interactions within the stratified analyses.

In conclusion, although there were associations between ghrelin, leptin, and either Barrett's esophagus or GERD symptoms, the current data do not fully explain how abdominal obesity increases the risk of Barrett's esophagus. Higher circulating ghrelin levels are associated with an increased risk of Barrett's esophagus; this association is likely independent of GERD but may be modified by GERD. This relationship requires further investigation as it could partially explain the biological mechanism linking obesity, GERD, and Barrett's esophagus. Leptin is associated with GERD symptoms, but is inversely associated with Barrett's esophagus, contrary to what has been reported in some previous studies. These inconsistent results highlight the need for further research into the pathophysiology linking ghrelin, leptin and GERD, as well as the impact of GERD treatment on these peptides. Future studies may also benefit from the evaluation of differences between different populations and from higher-powered analyses of strata for risk factors of interest, including strata of GERD symptoms and measures of obesity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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