Published in final edited form as:
Clin Gastroenterol Hepatol. 2013 August ; 11(8): 934–943. doi:10.1016/j.cgh.2013.02.017.

Association Between Markers of Obesity and Progression From Barrett's Esophagus to Esophageal Adenocarcinoma Adenocarcinoma

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Abstract

BACKGROUND & AIMS—Individuals with Barrett's esophagus (BE) have an increased risk of developing esophageal adenocarcinoma (EA). Obesity contributes to development of BE and its progression to cancer. We investigated the roles of obesity-induced hyperinsulinemia and dysregulation of adipokines in these processes.

METHODS—We measured fasting levels of glucose, insulin, leptin, and adiponectin in 392 patients enrolled in the Seattle BE Study. We calculated homeostatic model assessment (HOMA) scores (a measure of insulin sensitivity) and identified subjects with metabolic syndrome. We evaluated the association between these measures and risk of EA using Cox regression models adjusted for known risk factors.

RESULTS—Increasing HOMA scores were associated with increasing risk for EA; the strongest association was observed within the first 3 years after participants entered the study (hazard ratio (HR)=2.45; 95% confidence interval [CI], 1.43–4.1; P_{trend}=.001). Leptin level was also significantly associated with increased risk of EA within 3 y (HR=2.51; 95% CI 1.09–5.81; P_{trend} =0.03) and 6 y (HR=2.07; 95% CI 1.01–4.26; P_{trend}=0.048) of baseline. The level of high molecular weight adiponectin had a non-linear inverse association with risk of EA; the strongest

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Disclosure of Potential Conflicts of Interest

B.J. Reid is employed with Intergovernmental Personnel Act with NCI, which pays 20% of his salary, 7/1/09-present. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH. No potential conflicts of interest were disclosed by the other authors.

Author Contributions:

Catherine Duggan: analysis and interpretation of data; drafting of manuscript; Lynn Onstad: analysis and interpretation of data; critical revision of manuscript Sheetal Hardikar: analysis and interpretation of data; critical revision of manuscript Patricia L Blount and Brian J Reid: acquisition of data, critical revision of the manuscript Thomas L Vaughan: study concept and design; acquisition of data, drafting of manuscript

associations were observed in the second tertile (HR=0.34; 95% CI, 0.14–0.82). Metabolic syndrome was not associated with risk of EA.

CONCLUSIONS—Among patients with BE, increased levels of leptin and insulin resistance are associated with increased risk for EA, whereas increased level of high molecular weight adiponectin is inversely associated with EA. These biomarkers might be used to determine cancer risk among patients with BE.

Keywords

esophageal adenocarcinoma; Barrett's esophagus; obesity; anthropometry; adipokines; leptin; adiponectin; HOMA; insulin sensitivity; metabolic syndrome; insulin resistance; esophageal cancer; overweight; body mass; cancer risk factor

Introduction

Incidence of, and mortality from, esophageal adenocarcinoma (EA) has increased more than 5-fold in western populations in the past 4 decades,[1, 2] and have paralleled increasing prevalence of obesity in many countries.[3, 4] Overweight, measured by body mass index (BMI), is estimated to account for 36–41% of EA cases in the general population.[5, 6]

Barrett's esophagus (BE), a pre-malignant precursor to EA, also is associated with obesity.[7–11] It is characterized by replacement of a portion of normal stratified squamous epithelium of the lower esophagus with metaplastic columnar epithelium containing goblet cells. While individuals with BE experience a substantially increased risk of developing EA compared with the general population,[12] absolute incidence of EA is relatively low, with wide-ranging estimates from 0.1–0.7% per year.[13–16] Current methods of risk stratification, based largely on histology, are ineffective at accurately identifying the small subpopulation of BE patients who will progress to EA; thus most BE patients are often entered into a program of long-term endoscopic surveillance in the hope of identifying new cancers early when surgical treatment is most likely to affect a cure. Identifying a panel of biomarkers and other risk factors which accurately classify persons into risk categories would be of substantial clinical benefit, given the costs, risks and inconvenience of long-term surveillance.[14]

Overweight and obesity are associated with metabolic dysregulation including hyperinsulinemia and insulin resistance (measured by the Homeostatic Model Assessment (HOMA) score),[17] presence of the metabolic syndrome, and alterations in levels of adipokines, such as adiponectin and leptin, associated with increased risk of developing colon and other cancers.[18–22] Insulin stimulates cell proliferation in normal and malignant cell lines,[23] and can down-regulate IGF binding proteins, increasing bioavailable mitogens with accompanying downstream cellular effects.[24–27] The association between insulin and risk of EA is unknown. A recent meta-analysis reported an association with diabetes and risk of EA,[28] but the majority of studies included did not distinguish between type I and 2 diabetes.

Leptin, a regulator of appetite, is secreted predominantly by adipose tissue, with serum levels increasing in proportion to fat mass. It is associated with increasing cellular proliferation and decreasing apoptosis in EA cells in vitro.[29] Adiponectin is a peptide hormone with levels inversely correlated to BMI,[30] and has anti-diabetic and anti-inflammatory actions including stimulation of glucose utilization and inhibition of gluconeogenesis.[31, 32] It circulates as 3 oligomeric isoforms: high, medium and low molecular weight (HMW, MMW, LMW) adiponectin; the different isoforms display distinct

biological effects.[33] The HMW isoform is correlated more closely with insulin resistance and metabolic dysfunction that any of the other isoforms.[34]

Adiponectin, acting via its receptor, inhibits leptin-stimulated EA cell proliferation.[35] In some,[36–38] but not all studies,[39] plasma adiponectin levels were inversely associated with risk of BE.[36, 40]

Prospective cohort studies have observed that presence of the metabolic syndrome, a cluster of risk factors associated with increased risk of cardiovascular and diabetic morbidities, is also a predictor of colorectal cancer.[41, 42] It is unclear whether this can be accounted for by abdominal obesity or by associated dysregulation in glucose homeostasis or adipokine secretion. Whether altered levels of these markers, or presence of the metabolic syndrome are associated with increased risk of malignant progression to EA in patients diagnosed with BE is unknown.

Here, we evaluate whether low levels of total and High Molecular Weight (HMW) adiponectin, elevated levels of leptin, insulin resistance, and the presence of the metabolic syndrome, are associated with increased risk of developing EA in the Seattle Barrett's Esophagus Study (SBES), a prospective cohort of BE patients.

Methods

Study Setting, Participants, and Recruitment

The SBES is a prospective cohort of Barrett's esophagus patients established in 1983. Details of aims, study design and recruitment procedures have been published previously.[43, 44] This report is based on 427 participants with a diagnosis of BE and no history of esophageal cancer who participated in the study between February 1, 1995 and September 30, 2009. Inclusion in the cohort was dependent on a diagnosis of BE.

At the baseline visit (first visit on or after February 1, 1995) participants completed an extensive personal interview, underwent anthropometric assessments, gave a blood sample, and underwent an endoscopy. Participants attended scheduled follow-up visits where their information was updated and a repeat endoscopy with biopsies was performed. Follow-up assessments occurred at six month to two year intervals depending on the patients' risk of developing EA (based on histologic and flow cytometric assessments) with high-risk patients returning every six months. This study was approved by the Institutional Review Boards of the FHCRC and the University of Washington, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each subject.

Outcome Assessment

At baseline and follow-up visits, patients underwent endoscopies with multiple biopsies as described previously.[45–47] Briefly, four-quadrant biopsies were taken for histological evaluation at each 2 cm interval of the segment with columnar epithelium, and at 1 cm intervals for patients with a history of high-grade dysplasia. Biopsies were fixed, processed and reviewed by a single pathologist who was blinded to patient status. At each review participants were classified according to the most severe histological diagnosis (BE; low grade dysplasia; high grade dysplasia; EA). Development of EA in the Barrett's segment was considered as the end-point for these analyses, defined as invasion of neoplastic epithelium beyond the basement membrane of the esophageal mucosa into the surrounding lamina propria, muscularis mucosa or submucosa.[45, 46] Of the 427 participants, 411 had at least one follow-up endoscopy. Of these, 395 had available plasma samples which were

assayed for the analytes of interest. We excluded 3 participants with haemolzyed samples. The final sample is 392 participants.

Assays

Fasting blood samples were collected from patients at baseline, processed within 3 hours of collection, and stored at −80° C until analysis. Blind duplicate samples included in each batch were used to calculate intra- and inter-assay coefficients of variation (CV). The following is a list of the biomarkers that were measured from plasma, and their intra- and inter-assay CVs, respectively: Total and HMW adiponectin (Multimeric ELISA ALPCO Diagnostic, Salem, NH, USA), total adiponectin 3.8% and 6.4%; HMW adiponectin 5.0% and 8.7%; insulin (TOSOH auto analyzer; Tosoh Bioscience, Inc., San Francisco, CA, USA), 11.0% and 11.9%; glucose (Clinical Chemistry Autoanalyzer, using the glucose/hexokinase procedure), 1.4% and 1.4%; leptin (Linco Research Human Leptin RIA, Millipore, Billerica, MA, USA), 5.2% and 5.3%; high density lipoprotein (HDL; Clinical Chemistry Autoanalyzer using the HDL-C plus procedure, Roche Diagnostics), 3.3% and 3.3%; total trigyclerides (Roche Hitachi Modular P, Roche Diagnostics), 1.6% and 1.6%.

Covariates

A structured baseline interview collected detailed information on lifestyle exposures including smoking history; medical history including history and current use of various medications; and anthropometric measurements including height, weight, and circumference of waist, hips, thighs, and abdomen. All measurements were performed twice, and averaged. BMI was calculated as kg/m^2, and categorized according to WHO criteria[48] as $<25\ kg/m^2$; ≥ 25 and $<30\ kg/m^2$; and $\geq 30\ kg/m^2$.

Homeostatic model assessment (HOMA), first described in 1985,[17] is a method for assessing β-cell function and insulin resistance and is calculated as (insulin μU/mL × glucose mmol/L)/22.5. Metabolic syndrome is defined as a cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus (T2D). There are several definitions of metabolic syndrome, with considerable overlap. We used two; (1) The International Diabetes Federation (IDF) definition which requires the presence of BMI>30 kg/m^2 or waist circumference >102 cm (men) or >88 cm (women), in addition to 2 of the following: raised triglycerides ≥150 mg/dL; reduced HDL <40 mg/dL (men) or <50 mg/dL (women); elevated blood pressure (systolic > 130 mmHg; or diastolic >85 mmHg); elevated raised fasting glucose ≥100 mg/dL or previously diagnosed T2D; and (2) The American Heart Association (AHA)[49] definition, which requires the presence of 3 of the following criteria: Elevated waist circumference >102 cm (men) or >88 cm (women), raised triglycerides ≥150 mg/dL; reduced HDL <40 mg/dL (men) or <50 mg/dL (women); elevated blood pressure (systolic >130 mmHg; or diastolic >85 mmHg); and elevated raised fasting glucose ≥100 mg/dL or use of medication for hyperglycemia. We were unable to determine metabolic syndrome for 4 participants due to missing information.

Statistical Analysis

Differences in analytes between males and females were estimated using analysis of variance (ANOVA) and Pearson's Chi-square test for continuous and categorical variables, respectively. Pearson correlation coefficients were calculated to represent associations between continuous variables. Insulin, leptin, and adiponectin were not normally distributed, and were log-transformed. Hazard ratios (HR) for EA and their 95% confidence Intervals (CI) were based on partial likelihoods from Cox's proportional hazards models.[50] Tests of the proportional hazard assumption were carried out using Schoenfeld residuals, and held for all covariates tested.

The underlying time metric was calculated as the time elapsed between date of entry into the cohort, and either date of cancer diagnosis, or date of last follow-up. In subanalyses HRs were calculated for the first three and six years after baseline blood draw. The likelihood ratio test was used to estimate the effect of covariates on the HR. We selected the following covariates from available data for inclusion with the variable of interest: age (continuous); 3-category BMI (kg/m^2); sex (male/female); regular non-steroidal anti-inflammatory drug (NSAID) use at baseline (Yes/No); cumulative use of cigarettes (pack-years) at baseline. Covariates considered but not included in the final model (as they did not significantly change the likelihood ratio score) included race/ethnicity, and waist-hip ratio, categorized as quartiles. We also examined established risk factors and medication use, including proton pump inhibitors (PPI; ever/never use and cumulative use); presence/absence of hiatal hernia; alcohol use (categorized as ever/never and never/current/former), and duration (years) of alcohol use. Length of Barrett's segment (cm) and history of T2D were also examined.

We estimated the relationship between levels of adiponectin, leptin, HOMA scores and presence of metabolic syndrome, and development of EA adjusting for sex; age at baseline; BMI (3-category); cumulative use of cigarettes (pack-years) at baseline and regular NSAID use at baseline. P-values were estimated using the Wald test for trend. We also evaluated the associations with outcome in males only; numbers were too few to investigate the associations in women.

We also determined whether the association of the analytes with outcome was the same across subgroup categories, using a test of homogeneity and trend across groups, specifically: users/non-users of NSAIDs at baseline; BMI</≥25 kg/m^2; and regular/non-regular cigarette users at baseline. All p-values are two-sided. Analyses were performed using STATA 11 (Statacorp, TX USA).

Results

Characteristics of the study participants are shown in Table 1. Median follow-up time was 80.5 months. Mean age of participants was 61.0 years; mean BMI was 29.4 kg/m^2. The majority of the participants were male (81.9%) and white (96.4%), and 86.2% had a BMI≥25 kg/m^2. Twenty-four participants reported a history of diabetes. Of the 392 participants, 43 were diagnosed with esophageal adenocarcinoma during follow up.

Presence of abdominal obesity was significantly more common among females than males (P=0.003), although males had higher waist circumferences than females. Leptin, adiponectin, HDL and triglyceride levels were also significantly higher in females compared to males (Table 2). There was no significant difference between the sexes in insulin or glucose levels, HOMA scores or presence of the metabolic syndrome as defined by the AHA. However, significantly more females than males had the IDF-defined metabolic syndrome.

Leptin correlated strongly with BMI, and more weakly with HOMA, waist circumference, and HDL (Table 3). The correlation with HOMA scores was stronger in men than women. HOMA scores correlated positively with BMI and waist circumference, more weakly with triglycerides, and inversely with HDL levels. As expected, both total and HMW adiponectin correlated inversely with HOMA scores, waist circumference and BMI. While there was an inverse correlation between leptin and both total and HMW adiponectin in males and females, when combined there was no association between these variables.

Table 4 shows the associations between levels of total and HMW adiponectin, leptin, HOMA scores, presence of the metabolic syndrome and risk of developing esophageal

adenocarcinoma among all participants during all years of follow up and within the first three and six years after entry into the SBES cohort.

HOMA scores, analyzed as a log-transformed variable, were significantly associated with increased risk of progression to EA within three years (HR 2.45 95% CI 1.43–4.19), six years (2.06 95% CI 1.30–3.25) and over all follow up (HR=1.64 95% CI 1.08–2.48). Similar to HOMA scores, log-transformed leptin levels were more strongly associated with risk of developing EA when we restricted the analyses to within three years (HR=2.51 95% CI 1.09–5.81) and six years of baseline (HR=2.07 95% CI 1.01–4.26); but not when all events were included (HR=1.58 95% CI 0.83–3.00; Table 4). The associations observed between risk of EA and HOMA scores and leptin concentrations were stronger among men only, than among men and women combined (Table 4) Due to small numbers we were unable to investigate the association between outcomes in women only. The magnitude of the association between insulin and risk of progression was significant, and similar to, if smaller than, that of HOMA (data not shown).

Levels of HMW adiponectin in the second tertile were associated with a significantly reduced risk compared to the lowest tertile for all patients (HR=0.34, 95% CI 0.14–0.82), and for males only (HR=0.36 95% CI 0.15–0.88). The association did not reach statistical significance for patients diagnosed before 3- and 6 years had elapsed since entry into the cohort, but the magnitude of the association was similar. A somewhat attenuated and non-significant association was observed for total adiponectin. As there was evidence of a U-shaped association between adiponectin and cancer development, we did not model adiponectin as a continuous variable. We found no evidence that presence of the metabolic syndrome, defined either by the IDF or the AHA criteria, was associated with outcome (Table 4).

When we restricted the analysis to patients who did not have diabetes, log-transformed HOMA scores were no longer significantly associated with risk of EA, though the magnitude of the association was similar (HR=1.55 95% CI 0.96–2.51), but was significant for patients diagnosed with cancer less than 3 years (HR=2.55 95% CI 1.30–4.98) and 6 years (HR=2.06 95% CI 1.18–3.59) after entry into the cohort.

Sixty-nine participants were diagnosed with high grade dysplasia (HGD) at baseline. When this subgroup was analyzed separately, excess risk of EA was reduced by about half for HOMA, and completely eliminated for leptin at six years of follow up. In contrast, leptin (HR=3.95 95% CI 0.68–22.76) and HOMA scores (HR=5.00 95% CI 1.50–16.65) were strongly associated with progression to EA within six years in the 323 participants without HGD at baseline. However, confidence intervals were quite wide in this latter group, reflecting the low absolute risk of EA in the short-term after having been screened negative for HGD.

Exclusion of diabetic patients had no effect on results for HMW and total adiponectin, leptin or presence of the metabolic syndrome (data not shown). A history of T2D was not associated with risk of EA in all patients (HR=0.94 95% CI 0.22–3.99) or in males only (HR=1.05 95% CI 0.25–4.47) in fully adjusted models. Similarly, further adjustment of the models by presence/absence of hiatal hernia; PPI use (either cumulative use, or categorized as ever/never use), or BE segment length had no substantive effect on any of the models.

Among participants with BMI<25, increasing HOMA scores were associated with an approximate four-fold higher risk of EA than those with a BMI>25 (HR=6.31 95% CI 1.13–34.98 *vs.* HR=1.37 95% CI 0.88–2.12) with a non-significant but suggestive interaction term (P=0.09). It should be noted that confidence intervals were wide. There was no evidence of effect modification for other subgroups examined including NSAID vs. non-NSAID use at

baseline; and cigarette smokers vs. non–smokers at baseline. Risk associated with HMW adiponectin in the second tertile compared to the other two tertiles combined did not differ by subgroup. Finally, we examined if length of time of sample storage (time from blood collection to assay) had an effect on outcomes. There was no effect on any of the models when we included this variable (data not shown).

Discussion

In this study, we examined a variety of obesity-associated biomarkers and anthropometric measures and investigated whether they could predict the progression of BE to EA. We found that both an elevated HOMA score and increased leptin concentration measured in fasting plasma samples collected from patients diagnosed with BE was significantly associated with increased risk of progression to EA. These associations were strongest among males, within the first six years of follow up, and among those without HGD at baseline. Of note, the association observed in an age and sex-adjusted model was strengthened upon adjustment for BMI or waist circumference, along with cigarette pack years at baseline and regular NSAID use at baseline. Further adjustment for PPI use, presence of hiatal hernia, T2D, alcohol use and Barrett's segment length did not change the relative risk estimates in important ways.

To our knowledge this is the first demonstration of an association between HOMA scores and leptin and progression to EA in patients with BE. When we restricted the analysis to patients who did not have diabetes, HOMA scores were no longer significantly associated with risk of EA; this is likely due to a reduction in power, as the magnitude of the association was similar to that in the overall cohort. We were unable to examine risk by diabetes medication use, as detailed patterns of use were unavailable. While neither HOMA nor insulin levels have been studied as risk factors for progression to EA, a recent meta-analysis of 17 case-control studies and 11 cohort studies demonstrated that diabetic individuals had an increased risk of EA (summary relative risk 2.12, 95% CI 1.01–4.46) compared with non-diabetics.[28] Insulin may mediate its tumorigenic effects independently of adiposity: studies in a fatless transgenic mouse model, which develops T2D, displayed higher tumor incidence, tumor multiplicity, and decreased tumor latency compared to wild-type mice,[51] mediated via insulin, insulin receptor/IGF-1 receptor, and the PI3K/Akt pathway,[52] supporting the tumor-promoting effect of elevated circulating insulin levels.

Leptin is an adipokine which plays a central role in regulating food intake and energy expenditure.[53] It is elevated in the obese state,[54] suggesting that obese individuals are insensitive to the effects of leptin. Interestingly, administration of leptin normalizes fasting plasma glucose in obese type 2 diabetic rats, with a concomitant increase in JAK/STAT3 signaling, and an increase in inflammatory markers.[55] Leptin has mitogenic, anti-apoptotic and proliferative effects via phosphorylation of a variety of intracellular signaling pathways,[56] and stimulates proliferation and inhibits apoptosis in EA cells.[29] Leptin receptor expression in patients with EA was significantly associated with advanced tumor stage and nodal involvement.[57]

We also report an inverse association between HMW adiponectin levels and risk of developing EA, but only for levels of adiponectin in the second tertile compared to patients in the lowest tertile. Adiponectin is a peptide hormone that circulates as 3 oligomeric isoforms: high, medium and low molecular weight adiponectin, with levels inversely correlated to BMI;[30] and decrease in levels of the HMW isoform is correlated more closely with insulin resistance and metabolic dysfunction that any of the other isoforms.[34] It is downregulated in overweight and obesity, and upregulated under caloric restriction and weight loss [58] and in the lean state.[59–62] Adiponectin has anti-diabetic and anti-

inflammatory actions including stimulation of glucose utilization, fatty-acid oxidation and inhibition of gluconeogenesis.[31, 32] Overexpression of adiponectin in dyslipidemic, hyperglycemic and hyperinsulinemic leptin-deficient mice, rescued the diabetic phenotype, and reduced systemic inflammation and macrophage infiltration in adipose tissue.[32, 63–65] Additional antiinflammatory effects of adiponectin include reduction of phagocytic activity, reduction of TNFα secretion from macrophages,[66, 67] and suppression of TNFα-induced inflammatory changes in endothelial cells via inhibition of nuclear factor-κB phosphorylation, involved in cancer development.[68, 69] Several studies have assessed the activity of adiponectin on EA cell growth and proliferation: total adiponectin increases apoptosis and inhibits leptin-induced proliferation in EA cell lines.[35, 70] Low levels of adiponectin are associated with increased risk for a variety of obesity-related cancers,[33, 71] and with risk of developing BE,[36, 37] though the latter reported an association only with LMW adiponectin, and not with total adiponectin. Finally, serum adiponectin was significantly lower in 62 EA patients compared to healthy controls.[72]

Metabolic syndrome is characterized by elevated blood pressure, increased LDL/HDL ratio, and abdominal obesity. Several studies report an association between the presence of metabolic syndrome and risk of developing a variety of obesity-related cancers.[73–75] However it is unclear whether these merely reflect the underlying associations between risk and obesity itself. A BE case-control study found no difference between incidence of the metabolic syndrome using the IDF criteria, but using the NCEP definition, metabolic syndrome was significantly more common in the BE cohort.[76] However, our results did not demonstrate an association between the metabolic syndrome and risk of progression to EA.

Our study has several limitations. First, we have limited statistical power to identify moderate associations, as the analysis was based on only 43 cases. However our study represents the largest well-characterized cohort of BE patients reported in the literature, and we had sufficient power to detect associations with insulin, leptin and to a lesser extent, adiponectin. Nevertheless, we cannot rule out a weak association with metabolic syndrome. We measured biomarkers at only one time-point, and therefore cannot completely characterize participants' exposure to insulin for example. However, other studies indicate that a single fasting blood measure of these analytes is highly reproducible. We could not assess the effect of change in insulin resistance or other biomarkers, which would require testing interventions to change these analytes such as weight loss, physical activity, or medications to alter insulin resistance. Finally, we were unable to assess risk in specific subgroups, including women, due to small numbers.

In summary we report an association between HOMA scores (a measure of insulin resistance) and leptin concentration and risk of progression from BE to EA. These associations were stronger among males, those without HGD at baseline and during the initial 3–6 years of follow up. While further studies are required to confirm these findings we believe that the biological effects of these hormones, either directly, or via down-stream regulation of other growth factors, may mediate the effects of obesity on risk of EA. Insulin levels can be successfully lowered via behavioral/lifestyle interventions, such as increasing physical activity levels, and may represent a method of reducing risk of progression to EA.[77]

Acknowledgments

This project was funded through the following grants from the US National Institutes of Health, National Cancer Institute P01CA091955 and K05CA124911

Abbreviations

BE	Barrett's esophagus
BMI	Body Mass Index
CI	Confidence Interval
EA	Esophageal adenocarcinoma
HOMA	Homeostatic Model Assessment
HR	Hazard Ratio
HMW	High Molecular Weight
LMW	Low Molecular Weight
PPI	Proton Pump Inhibitors
SBES	Seattle Barrett's Esophagus Study

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Table 1

Baseline characteristics of the cohort

	All[1] 392	Male 321 (81.9%)	Female 71 (18.1%)
Race			
White[1]	378 (96.4%)	311 (96.9%)	67 (94.4%)
Other[2]	14 (3.6%)	10 (3.1%)	4 (5.6%)
Age			
Mean	61.0 (11.5)	60.8 (11.8)	61.8 (10.5)
Median	61.0	61.0	62.0
Range	30–87	30–87	39–83
P = NS [3]			
BMI (kg/m2)			
Mean (s.d)	29.4 (4.3)	29.3 (4.0)	30.0 (5.7)
Median	29.0	28.9	29.8
Range	17.4–48.6	20.3–47.3	17.4–48.6
<25	54 (13.8%)	41 (12.8%)	13 (18.3%)
≥25 & < 30	187 (47.7%)	162 (50.4%)	25 (35.2%)
≥30	151 (38.5%)	118 (36.8%)	33 (46.5%)
χ^2 = 5.56 P = 0.06[4]			
Waist (cm) baseline N=388			
Mean	101.1 (11.1)	102.4 (10.2)	95.3 (13.2)
Median	100.5	101.0	96.3
Range	66.0–155.9	78.5–155.9	66.0–125.7
P = 0.0001[3]			
Abdominal Obesity[5] N=388			
Normal	193 (49.5%)	169 (53.0%)	24 (33.8%)
Elevated[5]	197 (50.5%)	150 (47.0%)	47 (66.2%)
χ^2 = 8.54 P=0.003[4]			
Waist-hip ratio N=388			
Mean	0.95 (0.07)	0.96 (0.05)	0.87 (0.07)
Median	0.95	0.96	0.87
Range	0.72–1.14	0.84–1.14	0.72–1.03
P<0.0001[3]			
NSAID current use at Baseline N=391			
No	232 (59.3%)	183 (57.2%)	49 (69.0%)
Yes	159 (40.7%)	137 (42.8%)	22 (31.0%).
χ^2 = 3.34 P = 0.07[4]			

	All[1] 392	Male 321 (81.9%)	Female 71 (18.1%)
Smoking Status at baseline			
Never	143 (36.5%)	112 (34.9%)	31 (43.7%)
Former	209 (53.3%)	181 (56.4%)	28 (39.4%)
Current	40 (10.2%)	28 (8.7%)	12 (16.9%)
χ^2 = 8.17 P =0.02[4]			
Presence of Hiatal Hernia			
Yes	289 (74.1%)	232 (72.7%)	57 (80.3%)
No	90 (23.1%)	77 (24.1%)	13 (18.3%)
Unknown	11 (2.8%)	10 (3.1%)	1 (1.4%)
χ^2 = 1.92 P = 0.38[4]			
Length of Barrett's Segment at Diagnosis			
Mean (cm)	5.00	5.10	4.56
Median (cm)	4.00	4.00	4.00
P=0.15[3]	34 (8.7%)	29 (9.0%)	5 (7.0%)
Not measured (0)	79 (20.2%)	59 (18.4%)	20 (28.2%)
P=0.17[4]	279 (71.2%)	233 (72.6%)	46 (64.8%)

[1] 99.5% Non-Hispanic White (1 Hispanic, 1 Refusal)

[2] Black/African American N=1; Asian N=3; American Indian/Eskimo N=3, Other N=7

[3] Wilcoxan-Rank-sum

[4] Pearson Chi-squared

[5] Elevated Waist circumference defined as Females ≥ 88 cm; males: ≥ 102 cm.

Table 2

Distribution of Plasma Biomarkers and Components of the Metabolic Syndrome at Baseline

	All 392	Male 321 (81.9%)	Female 71 (18.1%)
Leptin (ng/mL)			
Mean	12.44	9.36	26.40
Median	9.45	7.70	23.70
Range (IQR)	1.90 – 63.6	1.90–45.00	2.80–63.60
P<0.0001 [1]			
Leptin (tertiles)			
Tertile 1	1.9–6.3	1.9–6.3	2.8–5.6
Tertile 2	6.4–12.4	6.4–12.4	7.3–12.0
Tertile 3	12.6–63.6	12.6–45.0	13.0–63.6
Total Adiponectin (μg/ml)			
Mean	5.33	4.93	7.06
Median	4.83	4.45	6.57
Range (IQR)	1.17–17.54	1.17–12.90	1.96–17.54
P<0.0001			
Total Adiponectin (tertiles)			
Tertile 1	1.17–3.84	1.17–3.84	1.96–3.79
Tertile 2	3.85–5.95	3.85–5.96	3.99–5.94
Tertile 3	5.97–17.54	5.96–12.90	6.32–17.54
HMW Adiponectin (μg/ml)			
Mean	2.19	1.94	3.30
Median	1.79	1.62	2.91
Range (IQR)	0.07–11.46	0.07–7.89	0.47–11.46
P<0.0001			
HMW Adiponectin (tertiles)			
Tertile 1	0.07–1.26	0.07–1.27	0.47–1.23
Tertile 2	1.27–2.49	1.27–2.45	1.30–2.39
Tertile 3	2.46–11.46	2.46–7.89	2.46–11.46
Glucose			
Mean	98.8	97.4	105.1
Median	93.0	93.0	93.0
Range	59.0–301.0	59.0–242.0	74.0–301.0
P = NS [1]			
HOMA Score			
Mean	2.36	2.38	2.22
Median	1.62	1.61	1.61

	All 392	Male 321 (81.9%)	Female 71 (18.1%)
Range	0.19–30.78	0.19–30.77	0.35–16.69
P = NS[1]			
HDL			
Mean	42.8	40.9	51.2
Median	41.0	39.0	47.0
Range	22.0–100.0	22.0–82.0	27.0–100.0
P <0.0001[1]			
Total Triglycerides			
Mean	160.3	156.1	179.3
Median	139.5	137.0	153.0
Range	38.0–989.0	38.0–965.0	62.0–989.0
P = 0.05[1]			
HDL			
Normal	190 (48.7%)	157 (49.2%)	33 (46.5%)
Reduced HDL *	200 (51.3%)	162 (50.8%)	38 (53.5%)
P=NS[1]			
Presence of Metabolic Syndrome (AHA)[N=388]			
No	199 (51.3%)	169 (53.3%)	30 (42.3%)
Yes	189 (48.7%)	148 (46.7%)	41 (57.7%)
χ^2 = 2.84 P = NS[1]			
Presence of Metabolic Syndrome (IDF) [N=388]			
No	204 (64.2%)	204 (64.2%)	35 (50.0%)
Yes	114 (35.8%)	114 (35.8%)	35 (50.0%)
χ^2 = 4.86 P = 0.03[1]			

[1]Wilcoxan-Rank-sum

Table 3

Pearson Correlation coefficients (r) between leptin, HOMA, adiponectin and other covariates in SEBS

	All N=392				Females N=71				Males N=321			
	Leptin	**HOMA**	**Total Adiponectin r**	**HMW Adiponectin**	**Leptin**	**HOMA**	**Total Adiponectin r**	**HMW Adiponectin**	**Leptin**	**HOMA**	**Total Adiponectin r**	**HMW Adiponectin**
Leptin	-				-				-			
HOMA	0.27[1]	-			0.21[3]	-			0.48[1]	-		
Total Adiponectin	0.07	−0.27[1]	-		−0.18[3]	−0.41[3]	-		−0.17[2]	−0.26[1]	-	
HMW Adiponectin	0.06	−0.26[1]	0.97[1]	-	−0.20[3]	−0.40[3]	0.97[1]	-	−0.19[2]	−0.25[1]	0.98[1]	-
Insulin	0.30	0.92	−0.32	−0.31	0.44	0.82	−0.46	−0.46	0.51	0.93	−0.31	−0.30
HDL	0.13[3]	−0.22[1]	0.55[1]	0.53[1]	−0.17	−0.42[3]	0.62[1]	0.58[3]	−0.08	−0.19[2]	0.42[1]	0.41[1]
Total Triglycerides	0.09	0.13[3]	−0.20[2]	−0.17[2]	0.02	0.13	−0.26[3]	−0.21[3]	0.07	0.15[3]	−0.23[2]	−0.22[2]
Age Waist	0.04	0.03	0.29[2]	0.29[2]	0.01	−0.12	0.24[3]	0.25[3]	0.04	0.05	0.33[1]	0.32[1]
Circumference (cm)	0.35[1]	0.37[1]	−0.28[1]	−0.28[1]	0.69[2]	0.43[2]	−0.23[3]	−0.22[3]	0.66[1]	0.38[1]	−0.21[2]	−0.23[2]
BMI kg/m^2	0.55[1]	0.42[1]	−0.31[1]	−0.33[1]	0.68[1]	0.48[1]	−0.40[3]	−0.41[3]	0.66[1]	0.42[1]	−0.33[1]	−0.35[1]

[1] P <0.0001

[2] P <0.001

[3] <0.01

Table 4

Adjusted associations between Leptin, HOMA scores, total and HMW adiponectin and the presence of the metabolic syndrome, and risk of developing EA

		3 Years[1] (23 events)			6 years[1] (32 events)			Full follow-up[1] (43 events)	
		HR	**95% CI**		**HR**	**95% CI**		**HR**	**95% CI**
Leptin Tertiles(ng/ml)									
1.90–6.30	5/133	1.00	Ref.	9/133	1.00	Ref.	14/133	1.00	Ref.
6.40–12.4	9/129	1.96	0.60–6.46	11/129	1.44	0.56–3.71	16/129	1.25	0.58–2.69
12.6–63.6	9/130	3.68	0.94–14.45	12/130	2.91	0.96–8.78	13/130	1.53	0.58–4.07
ln(Leptin)	23/392	**2.51**	**1.09–5.81**	**32/392**	**2.07**	**1.01–4.26**	43/392	1.58	0.83–3.00
P_{trend}			**0.03**			**0.048**			0.16
ln(Leptin) – Males only	22/321	**2.80**	**1.19–6.54**	29/321	**2.71**	**1.28–5.69**	39/321	**1.96**	**1.02–3.80**
P_{trend}			**0.02**			**0.009**			**0.045**
HMW Adiponectin									
0.07–1.26	10/131	1.00	Ref.	13/131	1.00	Ref.	18/131	1.00	Ref.
1.26–2.45	4/131	0.40	0.12–1.29	6/131	0.40	0.15–1.08	7/131	**0.34**	**0.14–0.82**
2.46–11.46	9/130	0.84	0.30–2.35	13/130	0.75	0.31–1.84	18/130	0.82	0.38–1.74
Total Adiponectin μg/ml									
1.17–3.84	10/1314	1.00	Ref	13/131	1.00	Ref	17/131	1.00	Ref
3.85–5.95	/131	0.41	0.13–1.35	6/131	0.41	0.13–1.10	9/131	0.46	0.20–1.05
5.95–17.54	9/130	0.87	0.31–2.41	13/130	0.78	0.32–1.90	17/130	0.83	0.38–1.80
HOMA Tertiles									
0.19–1.22	2/131	1.00	Ref.	7/131	1.00	Ref.	12/131	1.00	Ref.
1.24–2.15	8/131	4.51	0.89–22.83	10/131	1.63	0.59–4.52	14/131	1.13	0.51–2.54
2.15–30.78	13/130	**8.94**	**1.69–46.97**	15/130	**3.11**	**1.10–8.77**	17/130	1.72	0.75–3.98
ln(HOMA)	23/392	**2.45**	**1.43–4.19**	32/392	**2.06**	**1.30–3.25**	43/392	**1.64**	**1.08–2.48**
P_{trend}			**0.001**			**0.002**			**0.02**

		3 Years[1] (23 events)			6 years[1] (32 events)			Full follow-up[1] (43 events)	
		HR	**95% CI**		**HR**	**95% CI**		**HR**	**95% CI**
ln(HOMA) – Males only	22/392	**2.28**	**1.31–3.89**	29/321	**2.17**	**1.35–3.47**	39/321	**1.73**	**1.12–2.66**
P_{trend}		**0.003**			**0.001**			**0.01**	
Metabolic Syndrome (IDF)[4]									
No	14/239	1.00	Ref.	20/23912/	1.001	Ref.	26/239	1.00	Ref.
Yes	9/149	0.89	0.34–2.32	149	.05	0.45–2.42	17/149	1.14	0.56–2.36
Metabolic Syndrome (AHA)[2]									
No	11/199	1.00	Ref.	16/199	1.00	Ref.	23/199	1.00	Ref.
Yes	12/189	1.33	0.55–3.18	16/189	1.32	0.63–2.78	20/189	1.06	0.56–2.01

[1]Adjusted for age at baseline, sex, BMI (categorized as <25 kg/m2; ≥25 and <30 kg/m2; ≥30 kg/m2), cigarette pack years at baseline and regular NSAID use at baseline.

[2]Missing data for 4 participants. (AHA – American Heart Association, IDF- International Diabetes federation).