Title: Circulating Levels of Inflammatory and Metabolic Biomarkers and Risk of Esophageal Adenocarcinoma and Barrett's Esophagus: Systematic Review and Meta-Analysis

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Abstract

Background: Associations between circulating levels of obesity-related biomarkers and risk of esophageal adenocarcinoma (EAC) and Barrett's esophagus (BE) have been reported, but the results are inconsistent.

Methods: A literature search until October 2018 in MEDLINE and EMBASE was performed. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for associations between 13 obesity-related inflammatory and metabolic biomarkers and risk of EAC or BE using random-effect meta-analyses.

Results: Among 7641 studies, 19 were eligible for inclusion (12 cross-sectional, 2 nested case-control and 5 cohort studies). Comparing the highest versus lowest categories of circulating biomarker levels, the pooled ORs were increased for leptin (OR=1.68, 95% CI 0.95-2.97 for BE), glucose (OR=1.12, 95% CI 1.03-1.22 for EAC), insulin (OR=1.47, 95% CI 1.06-2.00 for BE), C-reactive protein (OR=2.06, 95% CI 1.28-3.31 for EAC), interleukin 6 (OR=1.50, 95% CI 1.03-2.19 for EAC), and soluble tumor necrosis factor receptor 2 (OR=3.16, 95% 1.76-5.65 for EAC). No associations were identified for adiponectin, ghrelin, insulin-like growth factor 1, insulin-like growth factor-binding protein 3, triglycerides, interleukin-8 or tumor necrosis factor alpha.

Conclusions: Higher circulating levels of leptin, glucose, insulin, C-reactive protein, interleukin 6 and soluble tumor necrosis factor receptor 2 may be associated with an increased risk of EAC or BE.

Impact: More prospective studies are required to identify biomarkers that can help select high-risk individuals for targeted prevention and early detection.

Introduction

The incidence of esophageal adenocarcinoma (EAC) has rapidly increased in Western populations (1), although the increase seems to have slowed down during recent years in some countries, e.g. in the United Kingdom and the Netherlands (2,3). The prognosis of EAC is poor with an overall 5-year survival <15-20% (1,4). There is a striking male predominance in EAC with the male-to-female incidence ratio of up to 9-to-1, for which the reasons remain unclear (1,5). Barrett's esophagus (BE), a replacement of the native squamous lining of the esophagus with a specialized columnar epithelium (metaplasia), is the precursor of EAC (1,6).

Gastroesophageal reflux disease (GERD) and obesity are the main risk factors for EAC and BE (1,4,6). GERD can damage the esophageal mucosa and lead to esophagitis, BE and subsequently EAC. Obesity, particularly central obesity (typical male fat distribution), may promote reflux through increased intra-gastric pressure and disruption of the gastroesophageal junction and the lower esophageal sphincter (7). Obesity is also a systemic disease that might increase EAC risk through other mechanisms, including chronic inflammation and metabolic alterations (6,8).

Despite these strong and readily assessable risk factors, it has been difficult to identify individuals with a high absolute risk of EAC enough to advocate endoscopic screening or surveillance. Circulating biomarkers could be a useful addition in this respect. Some studies have investigated associations between circulating levels of inflammatory and metabolic biomarkers and the risk of EAC or BE (9,10). The findings from the individual studies are not consistent, however, and whether associations differ between the sexes and contribute to the strong male predominance is unclear.

To clarify the role of a range of circulating levels of obesity-related inflammatory and metabolic biomarkers in the development and prediction of EAC and BE, we conducted a systematic review and meta-analysis.

Materials and Methods

Literature Search

A systematic search for studies published in MEDLINE and EMBASE databases from inception through October 2018 was conducted with no language restriction. The search strategy is presented in detail in Supplementary Table S1. Briefly, we used a combination of keywords for inflammatory and metabolic biomarkers and those for the outcomes EAC and BE to identify relevant publications. Candidate biomarkers were those that had been reported to be associated with obesity or cancer risk. The search terms for the biomarkers were predefined after a scoping search and referring to two previous systematic reviews on the topic (9,11). We also reviewed the reference lists of eligible original articles and the two previous systematic reviews to identify additional studies.

Study Selection

Studies meeting the following criteria were included: (1) cross-sectional, case-control or cohort studies in humans and published as original articles; (2) measuring the incidence of EAC or BE (rather than mortality) as an outcome; (3) examining associations between circulating levels of inflammatory or metabolic biomarkers and risk of EAC or BE; and (4) containing information necessary to estimate relative risk compared to a reference group and with a measure of precision (e.g. confidence interval [CI], standard error, variance, chi square and degree of freedom, or *P* value). In case of multiple reports on the same biomarker from the same study population, only the most recent or informative ones were included. Case-control studies in which biomarker

levels were measured at or after onset of the disease outcome were considered cross-sectional, because the temporal relation could not be determined.

Data Extraction and Study Quality Assessment

The following information was collected from the eligible studies into an electronic database by one researcher (SR) and independently checked by a second researcher (SHX): (1) study design and characteristics (first author, year of publication, study setting, follow-up period, number of participants by group, study population or comparison group, and verification of cases); (2) participants' age and sex; (3) examined biomarkers; (4) statistical analysis strategy (statistic model, covariates matched or adjusted for, and any stratified or sensitivity analysis); and (5) main findings. The quality of the included studies was independently assessed by two researchers (SHX and ENJ) and discrepancies were resolved by joint review of reports to reach consensus. The study quality was quantitatively scored according to the Newcastle-Ottawa Scale for assessing the quality of non-randomized studies in meta-analyses (12). This scale contains eight items which are categorized into three domains, i.e. selection, comparability, and assessment of exposure (case-control or cross-sectional studies) or outcome (cohort studies) of study participants, and the assessment provides a score ranging from 0 to 9, where higher scores indicate better quality.

Meta-Analysis

Meta-analyses were performed for associations between circulating biomarker levels and the risk of EAC, BE, and the combined outcome EAC or BE (hereafter labelled EAC/BE), whenever

possible. Pooled odds ratios (ORs) with 95% CIs were estimated using a random-effect model. The following 13 biomarkers, for which data were available in at least 2 studies, were examined: (1) adiponectin, (2) leptin, (3) ghrelin, (4) glucose, (5) insulin, (6) insulin-like growth factor 1 (IGF-1), (7) insulin-like growth factor-binding protein 3 (IGFBP-3), (8) triglycerides, (9) C-reactive protein (CRP), (10) interleukin 6 (IL-6), (11) IL-8, (12) tumor necrosis factor alpha (TNF-α), and (13) soluble tumor necrosis factor receptor 2 (sTNFR-2). Hazard ratios and risk ratios reported in cohort studies were used as proxies of OR, which was justified by the low incidence of EAC and BE in the population. For all biomarkers, except for glucose and triglycerides, we transformed effect sizes into a common scale of comparison before conducting the meta-analysis, i.e. comparing the highest versus lowest tertiles, under the assumption that the biomarker level is normally distributed and has a log-linear association with the outcome risk (13,14). The logarithm of OR for the highest versus lowest tertiles was estimated to be 1.27 times that for the top versus bottom halves, 0.86 times the highest versus lowest quartiles, 0.78 times the highest versus lowest quintiles, and 2.18 times that for per standard deviation increase. When studies reported risk estimates with different degrees of statistical adjustment for covariates, we used the fully-adjusted estimates. If multiple measures of obesity were employed, we used the risk estimates adjusted for waist-to-hip ratio, over waist circumference, and over body mass index. Four studies compared biomarker levels in BE patients separately with two control groups, i.e. GERD patients and another control group from the general population or patients undergoing colonoscopy screening (15-18). For these studies, we used the risk estimates in comparison with GERD patients in the main analyses. For the two most studied biomarkers, i.e. adiponectin and leptin, we conducted separate meta-analyses of risk estimates using different comparators, i.e. (1) GERD patients (for EAC or BE) or BE patients (for EAC) and (2) general

population or patients undergoing colonoscopy or upper endoscopy. We also conducted meta-analysis excluding one study which compared EAC patients with BE patients (19), on the combined outcome EAC/BE for adiponectin. We stratified the analyses by sex for adiponectin and leptin, but not for other biomarkers due to the limited number of available studies.

Statistical heterogeneity across studies was assessed by the I^2 statistic and Cochran's Q test. The I^2 statistics indicate the proportion of the total variance in risk estimates across studies that is due to heterogeneity rather than chance, and thus, the I^2 lies between 0% and 100%, with larger values indicating more heterogeneity (20). A *P* value <0.10 in the Q test was considered statistically significant, as conventionally used (21). Publication bias was assessed by visual inspection of funnel plots and the Begg's and Egger's tests (22,23).

The software Comprehensive Meta-Analysis version 3 (Biostat, Inc. Englewood, New Jersey) was used for all statistical analyses. All statistical tests were two-sided. We interpreted the results in terms of magnitudes of associations and precision of the risk estimates conveyed by 95% CIs, rather than using *P* values as measures of significance (24,25).

Results

Literature Search and Study Characteristics

The literature search identified 7641 studies. Among these, 19 studies fulfilled the inclusion criteria for meta-analysis, including 12 cross-sectional (15-18,26-33), 2 nested case-control (10,34), and 5 cohort studies (19,35-38). The full selection procedure of eligible studies is shown in Supplementary Figure S1. Fourteen of the eligible studies were conducted in the United States, 2 in Europe, and 1 was conducted in Australia, and the remaining 1 study included participants from both the United States and Europe. Eleven out of 12 cross-sectional studies measured circulating levels of biomarkers in BE patients compared with GERD patients, general population, or patients who had undergone endoscopy, while EAC was the outcome in the remaining 1 cross-sectional study. All nested case-control and cohort studies investigated associations between biomarker levels and EAC risk, either in the general population or in cohorts of BE patients. Characteristics of the included cross-sectional and nested case-control studies are presented in Table 1 and the cohort studies in Table 2.

A detailed study quality assessment is shown in Supplementary Tables S2 and S3. Briefly, most studies (14 out of 19) had overall quality scores ranging from 6 to 8, while 2 cross-sectional studies scored lower (4 or 5) and the remaining 1 cross-sectional study and 2 cohort studies scored higher (9). All included studies, except for the 2 cross-sectional studies with lower quality scores, performed well in terms of comparability, i.e. regarding controlling for confounding from the major risk factors for EAC and BE, i.e. age, sex, GERD, obesity and tobacco smoking.

Adipokines (Adiponectin, Leptin and Ghrelin)

Seven cross-sectional studies reported associations of circulating adiponectin levels with BE risk, and 1 nested case-control study and 1 cohort study reported associations with EAC risk. Meta-analysis showed no associations between the highest versus lowest tertiles of adiponectin levels and risk of BE (pooled OR=0.90, 95% CI 0.59-1.37), EAC (OR=0.87, 95% CI 0.60-1.25), or EAC/BE (OR=0.88, 95% CI 0.67-1.16) (Figures 1 and 2). Adiponectin levels were not associated with risk of EAC/BE in the meta-analysis when excluding the study comparing EAC patients with BE patients (OR=0.91, 95% CI -.65-1.27). There was a tendency of a decreased risk of EAC/BE associated with higher adiponectin levels in comparison with the general population or patients undergoing endoscopy (OR=0.79, 95% CI 0.55-1.12), but not in comparison with GERD or BE patients (OR=0.96, 95% CI 0.56-1.55) (Supplementary Figure S2). Stratified analysis by sex showed no associations between adiponectin levels and risk of EAC/BE either in men or in women (Figure 3).

Meta-analysis of 7 studies (including 1 pilot study and 1 validation study in the Australian study) found an increased risk of BE associated with higher leptin levels (OR=1.68, 95% CI 0.95-2.97, comparing the highest versus lowest tertiles) (Figures 1 and 2). An increased EAC risk was also reported in 1 cohort study (OR=1.53, 95% CI 0.58-4.05). Meta-analysis of these 8 studies generated similar estimates for the combined outcome EAC/BE (OR=1.64, 95% CI 1.01-2.68) (Figures 2). Stratified analysis by type of comparators showed that the association for leptin was restricted in comparison with the general population or patients undergoing colonoscopy screening (OR=1.90, 95% CI 1.29-2.81) rather than in comparison with GERD or BE patients (OR=0.97, 95% CI 0.49-1.90) (Supplementary Figure S3). The association between leptin levels and risk of EAC/BE was not substantially stronger in men (OR=2.00, 95% CI 0.96-4.16) than in women (OR=1.57, 95% CI 0.29-8.42) (Figure 3).

Associations between ghrelin levels and BE risk were reported only in 2 studies, providing a pooled OR of 0.93 (95% CI 0.33-2.63) (Figure 2 and Supplementary Figure S4).

Diabetes Biomarkers (Glucose, Insulin, IGF-1 and IGFBP-3)

Meta-analysis of 1 nested case-control study and 2 cohort studies showed a slightly increased risk of EAC associated with elevated glucose levels (OR=1.12, 95% CI 1.03-1.22) (Figure 2 and Supplementary Figure S5).

The pooled estimates of 5 cross-sectional studies showed that higher insulin levels were associated with an increased risk of BE (OR=1.47, 95% CI 1.08-2.00) and EAC/BE (OR=1.42, 95% CI 1.05-1.93, comparing the highest versus lowest tertiles) (Figure 2 and Supplementary Figure S6).

The meta-analysis of 2 cross-sectional studies and 1 cohort study showed a possibly decreased risk of EAC/BE associated with higher levels of IGF-1 (OR=0.60, 95% CI 0.31-1.16, comparing the highest versus lowest tertiles), while no such association was found for IGFBP-3 (Figure 2 and Supplementary Figure S6).

Triglycerides

Pooling of 3 cohort studies showed no association between triglycerides levels and EAC risk (OR=0.95, 95% CI 0.88-1.03, comparing the highest versus lowest categories) (Figure 2 and Supplementary Figure S7).

Inflammatory Biomarkers (CRP, IL-6, IL-8, TNF-α and sTNFR-2)

Meta-analysis of 2 studies showed an increased risk of EAC associated with higher CRP levels (OR=2.06, 95% CI 1.28-3.30, comparing the highest versus lowest tertiles), while no association between CRP levels and BE risk was found in the only identified study. An increased OR remained for the combined outcome EAC/BE (OR=1.43, 95% CI 1.02-2.01) (Figure 2 and Supplementary Figure S8).

Two studies reported an increased risk of EAC associated with higher levels of IL-6 (pooled OR=1.50, 95% CI 1.03-2.19, comparing the highest versus lowest tertiles), while no associations were found for IL-8 or TNF-α (Figure 2 and Supplementary Figures S8 and S9). Meta-analysis combining 3 studies showed an increased risk of EAC/BE associated with higher IL-6 levels (OR=1.58, 95% CI 1.12-2.22) (Figure 2 and Supplementary Figure S8).

Meta-analysis of 1 nested case-control study and 1 cohort study showed that higher pre-diagnostic sTNFR-2 levels were associated with an increased risk of EAC (OR=3.16, 95% CI 1.76-5.65) (Figure 2 and Supplementary Figure S9).

Other Biomarkers

Seven of the included studies also measured serum levels of some biomarkers except for the 13 biomarkers presented above. No meta-analysis was performed for these biomarkers because data were available in only one study for each of these. No associations were found for total cholesterol or high-density lipoprotein cholesterol (35,38). A cross-sectional study comparing 141 BE patients with 139 patients undergoing colonoscopy screening reported an increased risk of BE associated with higher levels of IL-12p70 and lower levels of IL-10 and IL-1β, while no

associations were found for interferon-γ (27). A cohort study of 397 BE patients found no associations between plasma levels of soluble tumor necrosis factor receptor 1 or F2-isoprostanes and risk of EAC (37). A recent case-control study nested in seven cohorts quantitated 69 circulating inflammation markers (using Luminex-based multiplex assays) in 296 EAC patients and an equal number of control participants. This study suggested an increased risk of EAC associated with higher levels of soluble IL-6 receptor, soluble vascular endothelial growth factor receptor 3, lipocalin-2, resistin, and serum amyloid A, and with lower levels of IL-3 and IL-17A (10).

Heterogeneity and Publication Bias

Measurements of heterogeneity across studies and publication bias for each biomarker are presented in Table 3. The heterogeneity tests suggested moderate to high heterogeneity across studies on adiponectin (I^2=45%, P=0.070) and leptin (I^2=70%, P=0.002). No evident publication bias was detected by the funnel plots (Supplementary Figure S10) or the Begg's and Egger's tests for these studies.

Discussion

This study indicates an increased risk of EAC or BE associated with higher circulating levels of some inflammatory and metabolic biomarkers, i.e. leptin, glucose, insulin, CRP, IL-6 and sTNFR-2. No associations were found for adiponectin, ghrelin, IGF-1, IGFBP-3, triglycerides, IL-8, or TNF-α.

Among strengths of the study is the extensive search strategy to identify all relevant publications covering a wide range of inflammatory and metabolic biomarkers. The harmonization of the reported associations on different scales of comparison into a common form enabled comparison of magnitudes of the associations for most of the studied biomarkers, and also more accurate assessment of heterogeneity and publication bias. There are also limitations. First, most of the included studies were cross-sectional in design, where biomarker levels were measured after the disease onset. Thus, reverse causality could not be ruled out. This should not be an issue for the findings for glucose, triglycerides, and sTNFR-2, however, because they were based on prospective studies. Second, no more than 5 studies were identified for most biomarkers, except for adiponectin and leptin. Third, substantial heterogeneity across studies was observed, probably due to the combination of different outcomes and types of comparators. We conducted meta-analyses for the three different outcomes, i.e. EAC, BE, and the combined outcome EAC/BE, whenever possible. We also stratified the analyses for adiponectin and leptin by type of comparators and sex, but this was not possible for other biomarkers due to the limited number of available studies. Finally, the measurement of biomarker levels was based on a single sample only, even in the prospective studies, making it impossible to assess any influence of longitudinal changes of biomarker levels on their associations with EAC or BE risk.

To the best of our knowledge, the present study is the first to systematically summarize evidence for the other examined biomarkers in relation to the risk of EAC or BE, except for the biomarkers leptin, insulin, and adiponectin. The findings of an increased BE risk associated with higher levels of leptin and insulin and no association with adiponectin are consistent with a previous meta-analysis (9). However, two more recent publications were added in this analysis, and the earlier meta-analysis did not assess the associations of these 3 biomarkers with EAC risk. Specifically, for the association between leptin levels and BE risk, the newly added study in this updated meta-analysis found a decreased BE risk associated with higher leptin levels (17), which differed from the earlier studies. Such inconsistency might be due to heterogeneity across studies in characteristics of study population (e.g. sex composition), type of comparators, quantitative assay for leptin levels, ascertainment of BE patients and adjustment for confounders, or chance. However, the limited number of existing studies precluded exploring potential sources of heterogeneity, e.g. using a meta-regression approach. More large-scale studies are needed to examine whether the association between leptin levels and BE risk varies across populations and strata of other factors, including sex, reflux and obesity.

Leptin is an adipokine secreted by adipose tissue and is involved in the regulation of energy balance, suppressing food intake and thereby inducing weight loss (39). The circulating levels of leptin positively correlate with the amount of body fat and are also influenced by sex hormones and some other inflammatory cytokines (40). Higher leptin levels have been linked with increased risk of several cancers, including breast, endometrial, colorectal and prostate cancers. The carcinogenic mechanisms associated with elevated leptin levels may include enhanced cell proliferation, changes in the regulation of certain cell signaling pathways, and promotion of inflammation and angiogenesis (41-43). Our meta-analysis of 3 prospective studies found that

elevated glucose levels were associated with a modestly (12%) increased risk of EAC, which was in line with a recent pooled study of 13 population-based studies showing a 30% increased risk of EAC or esophagogastric junctional adenocarcinoma in diabetes patients (44). A stronger association between insulin levels and BE risk was indicated, but the evidence was mainly based on cross-sectional studies and the association was attenuated after adjustment for leptin (26).

The associations between higher levels of CRP, IL-6 and sTNFR-2 and risk of EAC or BE support a role of systemic inflammation in the development of EAC, which may be a mechanism underlying the associations of obesity and tobacco smoking with EAC. A mediation analysis indicated that sTNFR-2 accounted for 33% of the association between central obesity (measured by waist circumference) and EAC risk (10). Taken together, the available evidence suggests that systemic inflammation and metabolic disorders may be pathways in the etiology of EAC.

The striking male predominance in EAC seems not to be explained by the two major risk factors, i.e. GERD and general obesity, given the similar exposure prevalence and strengths of associations of these factors with EAC risk between the sexes (1,5). Abdominal obesity, however, which is more common in men than in women, may contribute to the sex difference in EAC. Our findings in this study did not support stronger associations with adiponectin or leptin levels in men than in women, but large prospective investigations including more particpants of both sexes are needed to clarify this question. Sex hormonal factors may play a role in the etiology of EAC and BE (45,46), but the existing evidence is limited (1,5). If the role of sex hormones in the etiology of EAC is confirmed, an underlying mechanism may be the influence of sex hormones on inflammation, e.g. the anti- or pro-inflammatory effects of certain sex hormones depending on the biological microenvironment, which may subsequently lead to altered cancer risk.

Despite decades of efforts to develop the treatment, the overall prognosis in EAC remains poor, mainly because most patients are diagnosed at an advanced tumor stage (1,4). Earlier tumor detection, particularly among individuals at high absolute risk, has the potential to reduce the mortality from this cancer. A few risk stratification models have been developed for EAC and BE, showing promising performance (47-51). These models mainly combine clinical and lifestyle risk factors which are easily captured through questionnaires or medical records, while genetic biomarkers have thus far not improved the identification of high-risk individuals (50). Whether inclusion of a panel of inflammatory and metabolic biomarkers increases the accuracy of risk stratification models needs to be evaluated. However, because most of the identified biomarkers in this study showed modest associations with EAC or BE risk and are also associated with the major risk factors for EAC and BE, the addition of these biomarkers may improve the model performance only to a limited extent. Interestingly, this study found an over 3-fold increased risk of EAC or BE associated with higher pre-diagnostic sTNFR-2 levels, which particularly warrants further investigations. Nevertheless, any use of circulating biomarkers to identify high-risk individuals who may benefit from screening or surveillance should be scientifically justified before they may be tested in routine clinical practice.

In summary, this systematic review and meta-analysis suggests an increased risk of EAC or BE associated with higher circulating levels of some inflammatory and metabolic biomarkers, i.e. leptin, glucose, insulin, CRP, IL-6 and sTNFR-2. The available studies were too few to examine sex-specific associations for these biomarkers. More prospective studies are required to identify biomarkers that can help select individuals at high absolute risk of EAC for targeted prevention and early detection.

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Table 1. Characteristics of cross-sectional and nested case-control studies identified in a systematic search and included in the meta-analysis

Study	Country	Study period	Number of cases/controls	Age, years	Sex, males %	Source of controls	Outcome	Biomarkers
Rubenstein 2008	United States	N.A.	50/50	18-79, mean 60	Cases 80%, controls 54%	Patients for upper endoscopy	BE	Adiponectin, C-reactive protein
Kendall 2008	Australia	2003-2006	Pilot: 51/67; Validation: 306/309	18-79	Pilot: cases 51%, controls 51%; Validation: cases 68%, controls 67%	General population	BE	Adiponectin, leptin
Rubenstein 2009	United States	2002-2007	112/199	Cases: mean (SD) 57.4 (11.3); controls: 50.7 (13.3)	Cases 65%, controls 35%	GERD patients	BE	Adiponectin
Thompson 2010	United States	1997-2000	177/173	20-80	Cases 60%, controls 64%	General population	BE	Adiponectin, leptin
Greer 2012	United States	2005-2009	135 /932+135	Cases: mean (SD) 63.7 (11.2); colonoscopy screening controls: 54.5 (8.9); GERD controls: 56.4 (11.1)	Cases 80%, colonoscopy screening controls 35%, GERD controls 60%	Patients for colonoscopy screening; GERD patients	BE	Insulin, insulin growth factor 1, insulin-like growth factor-binding protein 1, insulin-like growth factor-binding protein 3
Rubenstein 2013	United States	N.A.	150/751	Cases: mean (SD) 61 (6.6); controls: 58.5 (6.7)	All men	Patients for colonoscopy screening	BE	Leptin, insulin, ghrelin
Greer 2013	United States	N.A.	10/65	Mean (SD) 64.7 (11.8)	80%	BE patients	EAC	Insulin, insulin growth factor 1, insulin-like growth factor-binding protein 1, insulin-like growth factor-binding protein 3

Garcia 2014	United States	2008-2011	141/139	Cases: mean (SD) 62.8 (6.7); controls: 61.2 (7.6)	Cases 97%, controls 97%	Patients for colonoscopy screening	BE	Adiponectin, leptin, insulin, 7 cytokines
Almers 2015	United States	2002-2005	284 /285+294	Cases: mean (SD) 62 (10.7); population controls: 62 (10.2); GERD controls: 62 (10.7)	Cases 73%, population controls 68%, GERD controls (69%)	General population, GERD patients	BE	Adiponectin
Greer 2015	United States	2005-2009	135 /1157+133	Cases: mean (SD) 63.7 (11); colonoscopy screening controls: 54.6 (8.8); GERD controls: 65.4 (11.1)	Cases 80%, colonoscopy screening controls 35%, GERD controls 40%	Patients for colonoscopy screening, GERD patients	BE	Adiponectin, leptin
Thomas 2016	United States	2002-2005	300 /290+296	Cases: mean (SD) 62 (11); population controls: 62 (10); GERD controls: 62 (11)	Cases 73%, population controls 68%, GERD controls 69%	General population, GERD patients	BE	Leptin, ghrelin
Di Caro 2016	United Kingdom	2011-2013	250/224	Cases: mean (SD) 63.8 (12.4); controls: 52 (16.4)	Cases 77%, controls 41%	Patients for upper endoscopy	BE	Glucose, insulin, cholesterol, triglycerides, high-density lipoprotein
Drahos 2017	United States	2003-2009	3167/15835	Mean (SD) 78.0 (6.5)	78%	General population	EAC	Glucose, triglycerides
Cook 2019	United States, 10 European countries	N.A.	296/296	Mean age at baseline 63.4	77%	General population	EAC	69 inflammation markers

BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; GERD, gastroesophageal reflux disease; SD, standard deviation; N.A., not available.

Table 2. Characteristics of cohort studies assessing associations between circulating biomarkers and risk of esophageal adenocarcinoma

Study	Country	Study period	Number of participants	Follow-up, years	Age at entry, mean (standard deviation)	Sex, males %	Study population	Biomarkers
Siahpush 2007	United States	1995-2003	344	Median 5.4	61.6 (11.7)	81%	Barrett's esophagus patients	Insulin growth factor 1, insulin-like growth factor-binding protein 3
Duggan 2013	United States	1995-2009	392	Median 6.7	61 (11.5)	82%	Barrett's esophagus patients	Adiponectin, leptin, glucose, triglycerides, high-density lipoprotein
Lindkvist 2014	Austria, Norway, Sweden	1972-2006	578700	Mean 12	44 (11.7)	50%	General population	Glucose, cholesterol, triglycerides
Hardikar 2014	United States	1995-2009	397	Median 6.14	61.2	81%	Barrett's esophagus patients	C-reactive protein, interleukin 6, soluble tumor necrosis factor receptors, F2-isoprostanes
Lin 2015	Norway	1994-2010	192903	Mean 10.6	49.5 (15.7)	48%	General population	Glucose, triglycerides, high-density lipoprotein

Table 3. Measurements of heterogeneity across studies and publication bias

Biomarker	Number of studies	Heterogeneity		Publication bias *	
		I^2	Q test	Begg's test	Egger's test
Adiponectin	9	45	P=0.070	P=0.677	P=0.292
Leptin	8	70	P=0.002	P=0.216	P=0.163
Ghrelin	2	82	P=0.010	-	-
Glucose	3	0	P=0.979	P=0.602	P=0.599
Insulin	5	0	P=0.597	P=0.327	P=0.159
Insulin-like growth factor 1	3	35	P=0.213	P=0.602	P=0.867
Insulin-like growth factor-binding protein 3	3	0	P=0.390	P=0.602	P=0.492
Triglycerides	3	0	P=0.579	P=0.602	P=0.343
C-reactive protein	3	61	P=0.080	P=0.118	P=0.063
Interleukin 6	3	0	P=0.551	P=0.118	P=0.038
Interleukin 8	2	85	P=0.010	-	-
Tumor necrosis factor alpha	2	0	P=0.650	-	-
Soluble tumor necrosis factor receptor 2	2	0	P=0.330	-	-

* Publication bias was assessed for meta-analyses of at least 3 studies only.

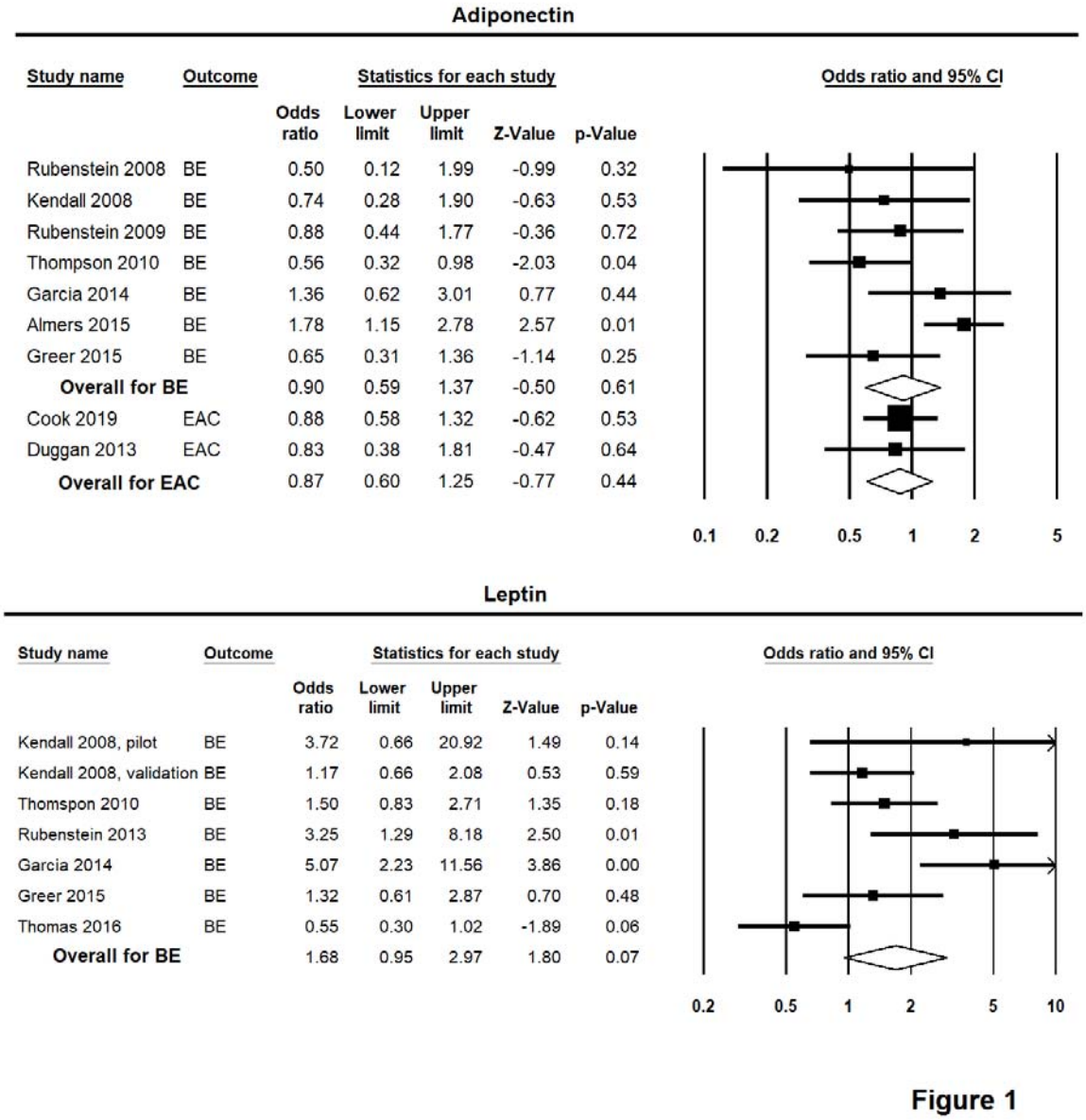


Figure. 1. Associations between adipokines levels and risk of esophageal adenocarcinoma or Barrett's esophagus

Forest plots for the associations between circulating adiponectin and leptin levels and risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE), expressed as odds ratio and 95% confidence interval (CI) comparing the highest with the lowest tertiles.

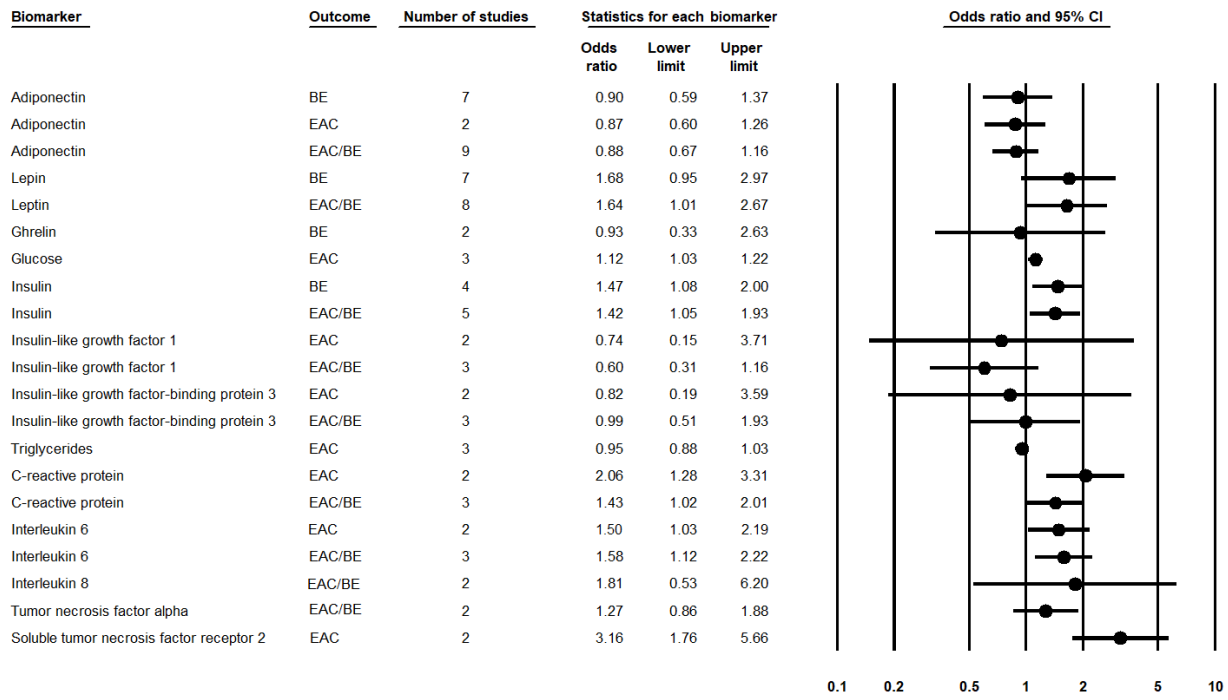


Figure. 2. Associations between biomarker levels and risk of esophageal adenocarcinoma or Barrett's esophagus

Associations between circulating levels of inflammatory and metabolic biomarkers and risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE), expressed as odds ratio and 95% confidence interval (CI) comparing the highest with the lowest tertiles.

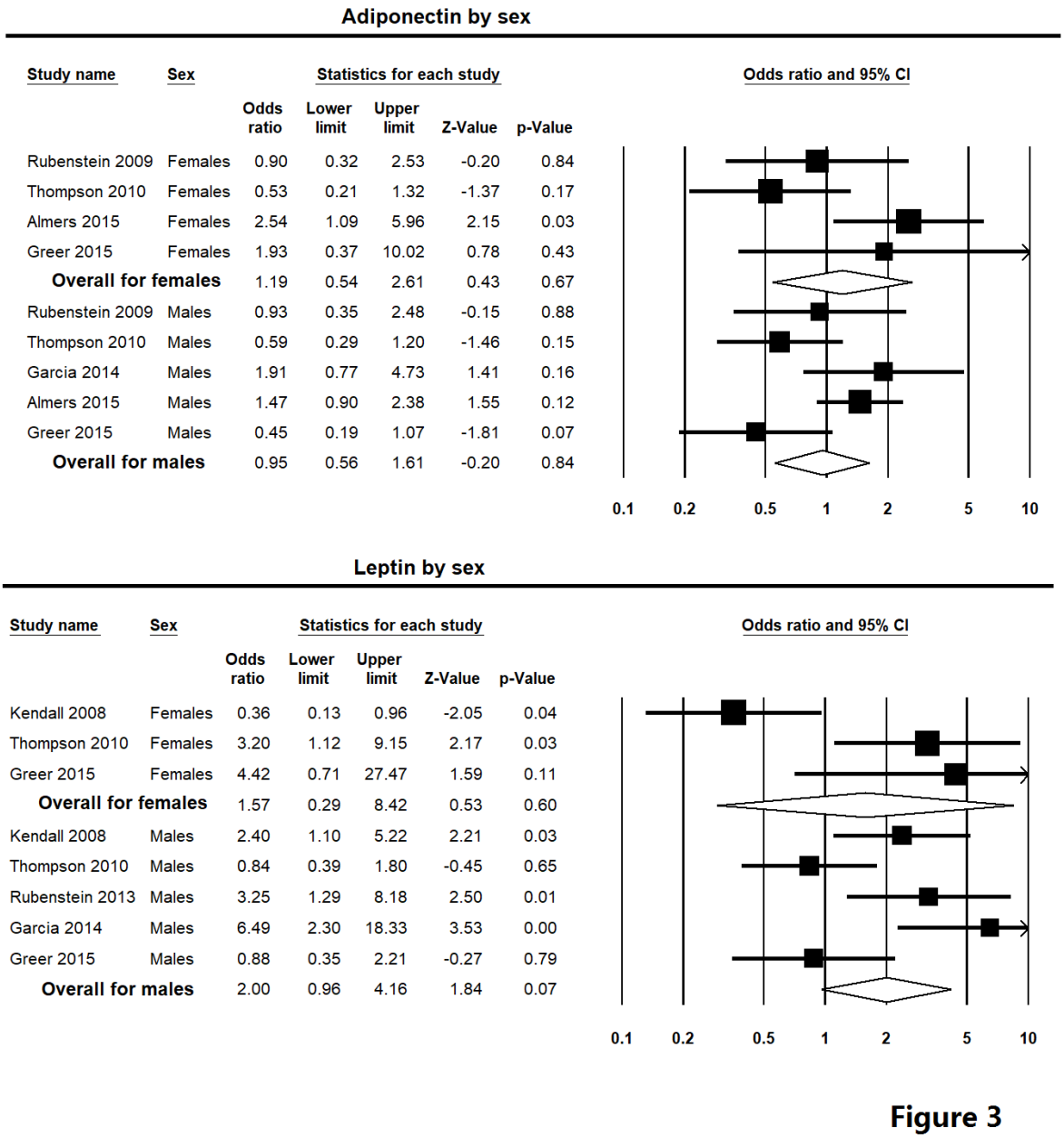


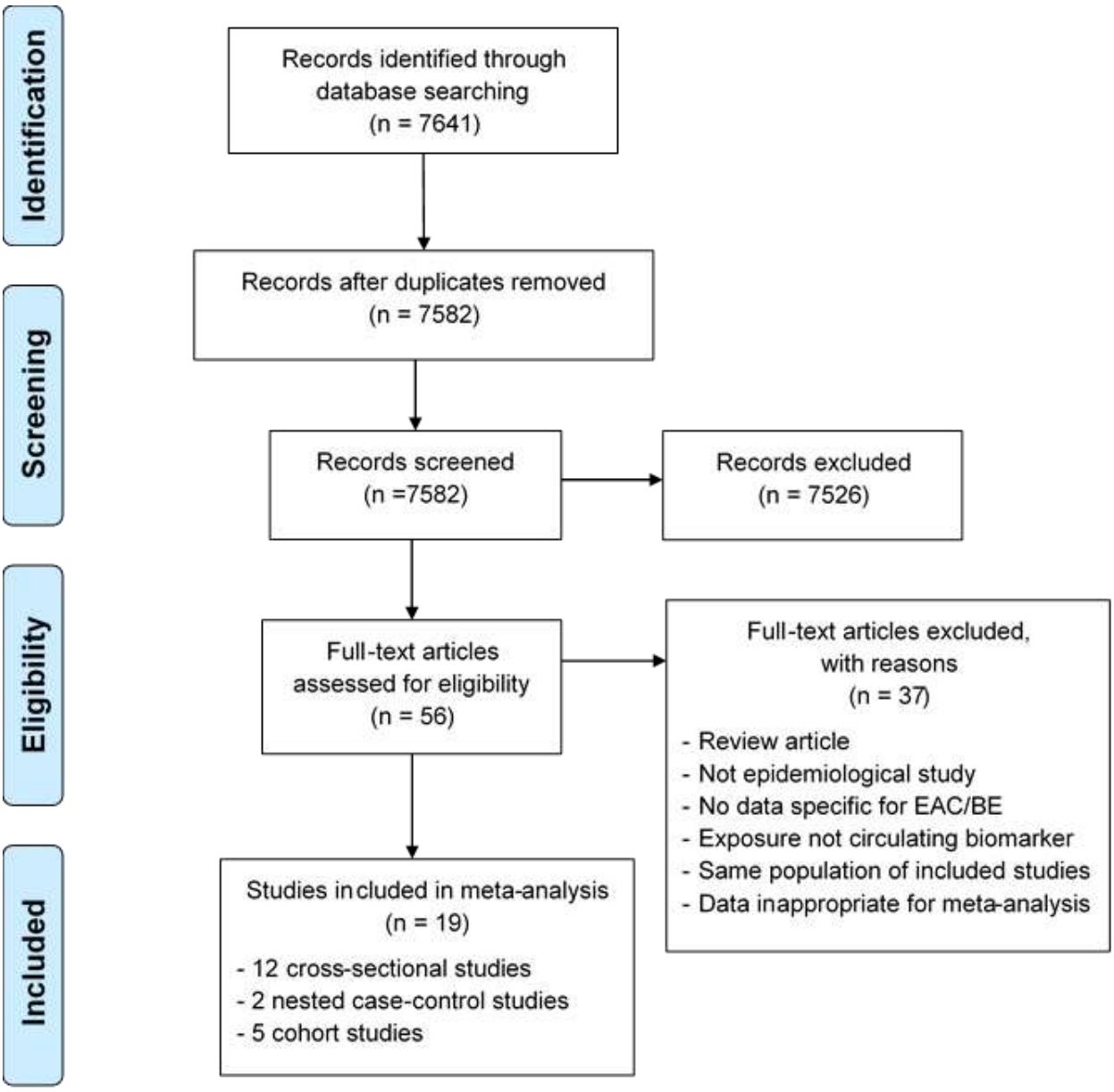
Figure. 3. Sex-specific associations between adipokines levels and risk of esophageal adenocarcinoma or Barrett's esophagus

Forest plots for the sex-specific associations between circulating adiponectin and leptin levels and risk of esophageal adenocarcinoma or Barrett's esophagus, expressed as odds ratio and 95% confidence interval (CI) comparing the highest with the lowest tertiles.

Supplementary Table S1. Literature search strategy

Step	Key words
1	lipid OR cholesterol OR HDL OR LDL OR triglyceride OR glucose OR insulin OR IGF OR C-reactive protein OR CRP OR adipocytokine OR adipokine OR leptin OR adiponectin OR resistin OR apelin OR interleukin-6 OR IL-6 OR interleukin-2 OR IL-2 OR (tumor necrosis factor) OR TNF OR (retinol binding protein) OR RBP4 OR chemokine OR (angiopoietin-like protein) OR interleukin* OR cytokine* OR metabolism OR metabolic OR inflammation OR inflammatory
2	esophagus OR oesophagus OR esophageal OR oesophageal
3	cancer OR carcinoma OR adenocarcinoma OR malignan* OR neoplas* OR tumour OR tumor OR Barrett OR Barrett's
4	1 AND 2 AND 3

All terms were searched on ABSTRACT in MEDLINE and on TITLE/ABSTRACT in EMBASE.



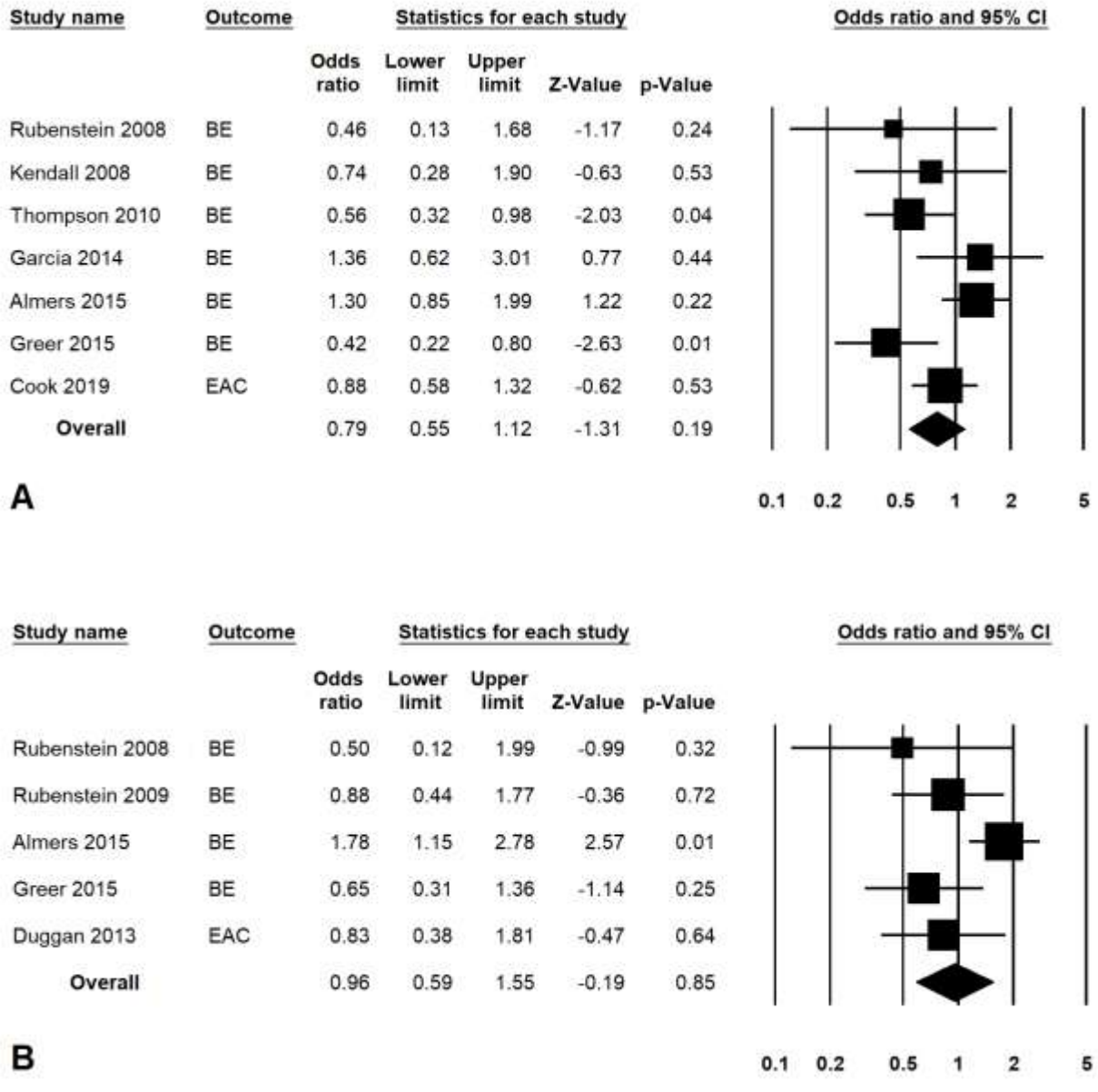
Supplementary Figure S1. Flow chart of selection of eligible studies

Supplementary Table S2. Quality Assessment of the cross-sectional and nested case-control studies according to Newcastle-Ottawa Quality Assessment Scale

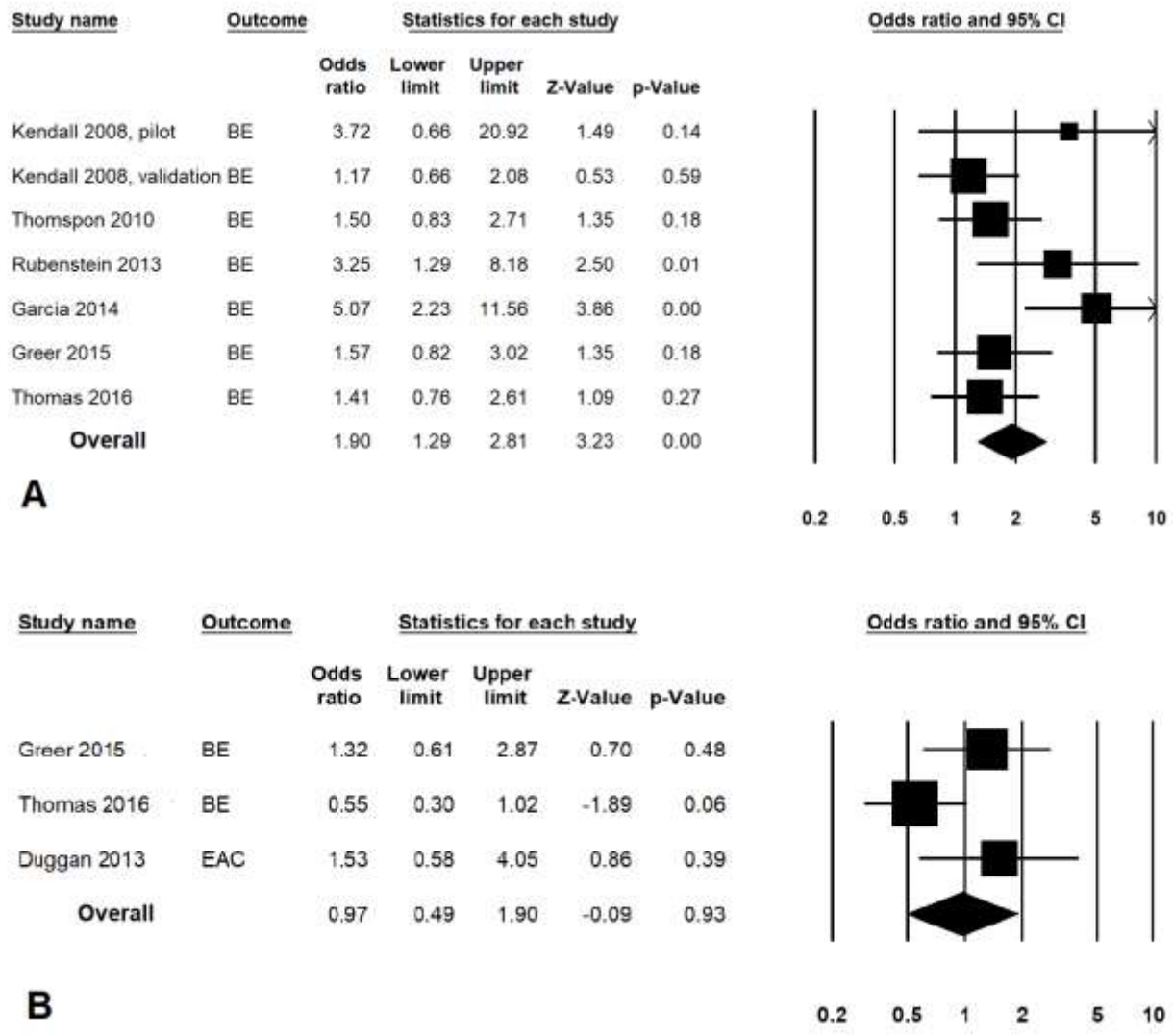
Study	Selection				Comparability	Exposure			Total
	Case definition	Representativeness of cases	Control selection	Control definition		Exposure ascertainment	Same method	Response rate	
Cross-sectional studies									
Rubenstein 2008	1	0	0	1	2	1	1	0	6
Kendall 2008	1	1	1	1	2	1	1	1	9
Rubenstein 2009	1	0	0	1	2	1	1	1	7
Thompson 2010	1	1	1	0	2	1	1	1	8
Greer 2012	1	0	0	1	2	1	1	0	6
Rubenstein 2013	1	1	0	1	2	1	1	0	7
Greer 2013	1	0	0	1	0	1	1	0	4
Garcia 2014	1	0	0	1	2	1	1	0	6
Almers 2015	1	1	1	1	2	1	1	0	8
Greer 2015	1	0	0	1	2	1	1	0	6
Thomas 2016	1	1	1	1	2	1	1	0	8
Di Caro 2016	1	1	0	1	0	1	1	0	5
Nested case-control studies									
Drahos 2017	0	1	1	1	2	0	1	0	6
Cook 2019	0	1	1	1	2	1	1	0	7

Supplementary Table S3. Quality Assessment of the cohort studies according to Newcastle-Ottawa Quality Assessment Scale

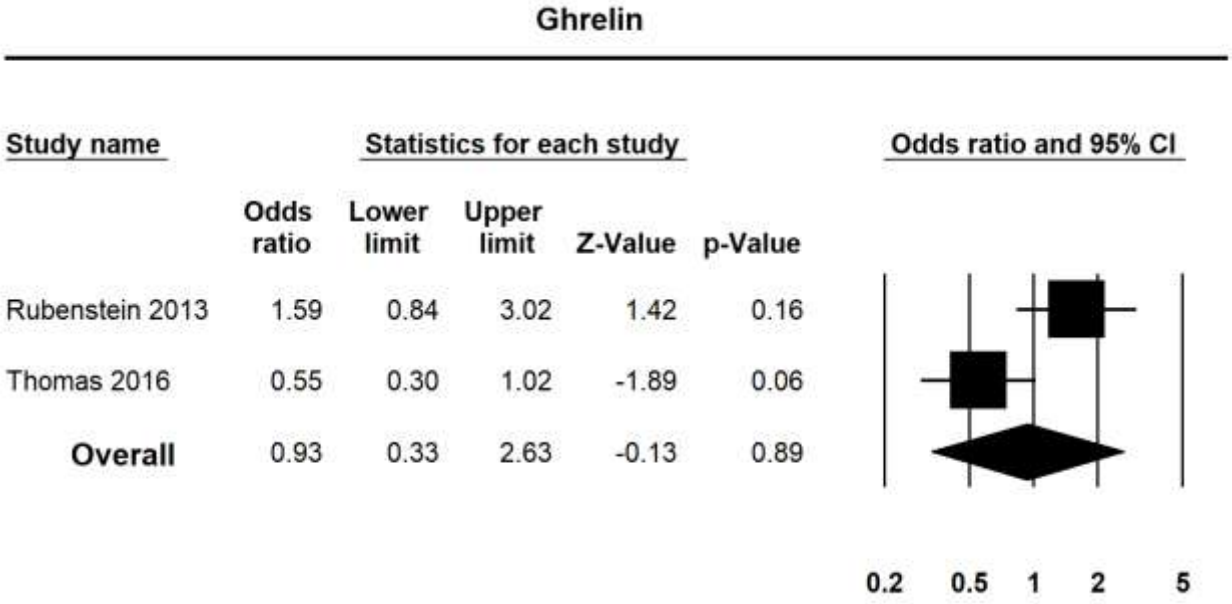
Study	Selection				Comparability	Outcome			Total
	Representativeness of exposed	Selection of non-exposed	Exposure ascertainment	Outcome not present at start		Outcome ascertainment	Follow-up duration	Lost to follow-up	
Siahpush 2007	0	1	1	1	2	1	1	0	7
Duggan 2013	0	1	1	1	2	1	1	1	8
Lindkvist 2014	1	1	1	1	2	1	1	1	9
Hardikar 2014	0	1	1	1	2	1	1	1	8
Lin 2015	1	1	1	1	2	1	1	1	9



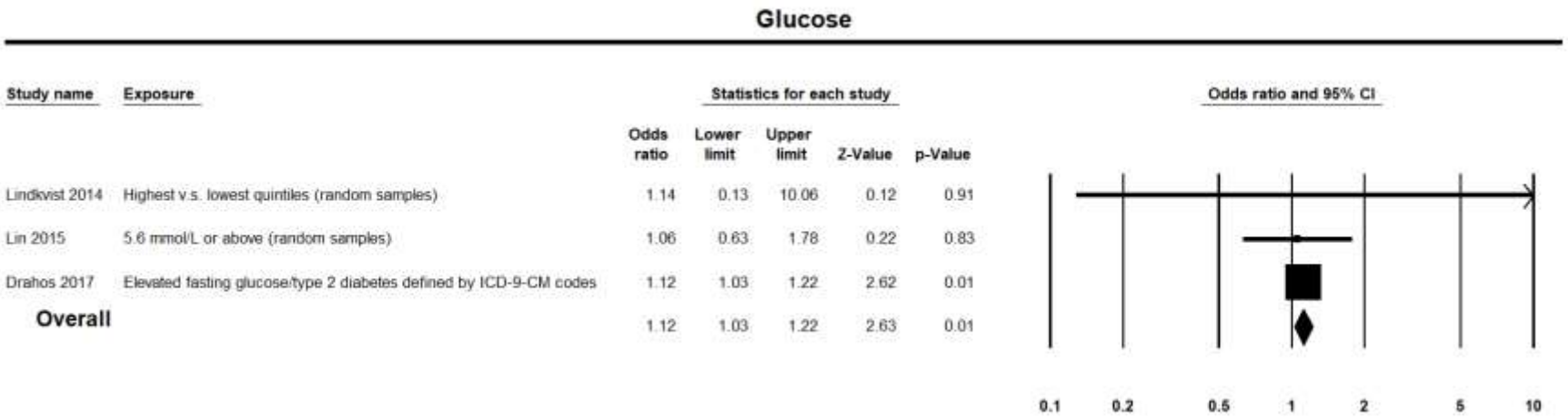
Supplementary Figure S2. Forest plot for the association between circulating adiponectin levels and risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE) in comparison with general population or endoscopy patients (A) and in comparison with patients with gastroesophageal reflux disease or Barrett's esophagus (B), comparing the highest versus lowest tertiles. CI: confidence interval.



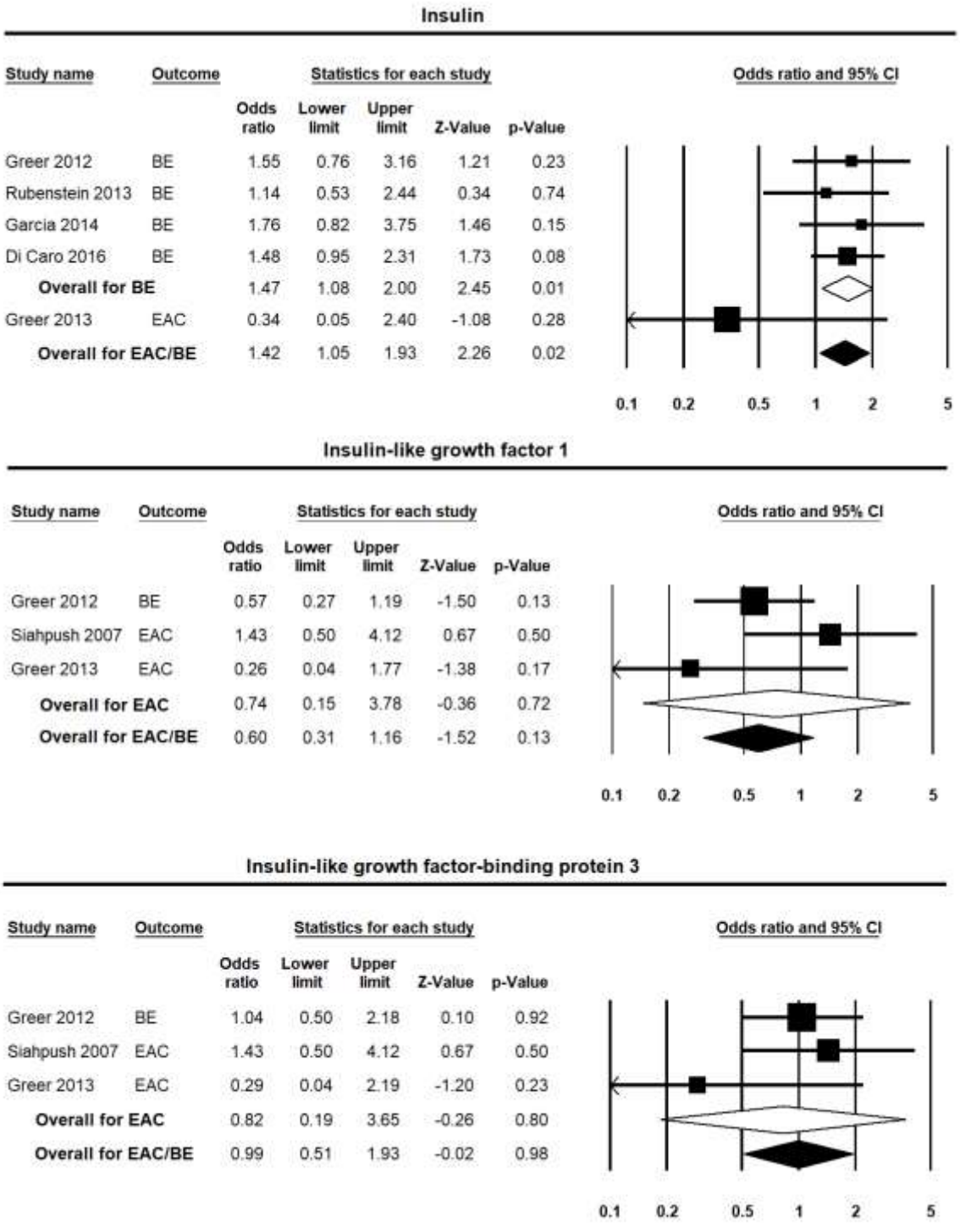
Supplementary Figure S3. Forest plot for the association between circulating leptin levels and risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE) in comparison with general population or endoscopy patients (A) and in comparison with patients with gastroesophageal reflux disease or Barrett's esophagus (B), comparing the highest versus lowest tertiles. CI: confidence interval.



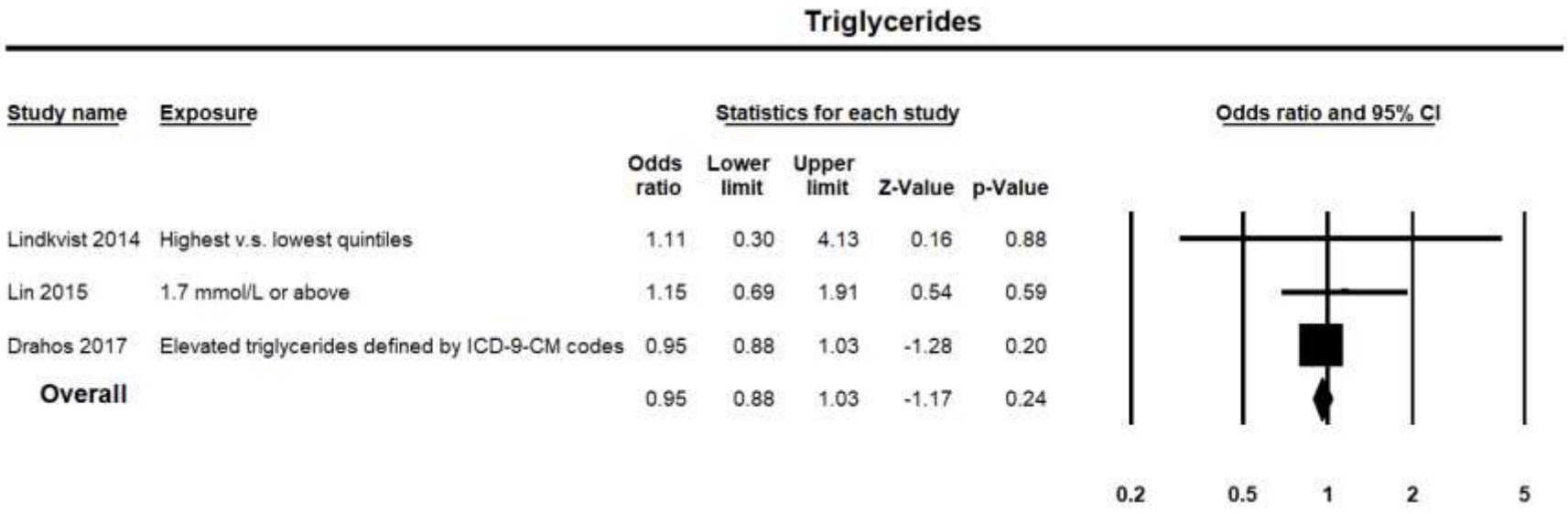
Supplementary Figure S4. Forest plot for the association between circulating ghrelin levels and risk of Barrett's esophagus, comparing the highest versus lowest tertiles. CI: confidence interval.



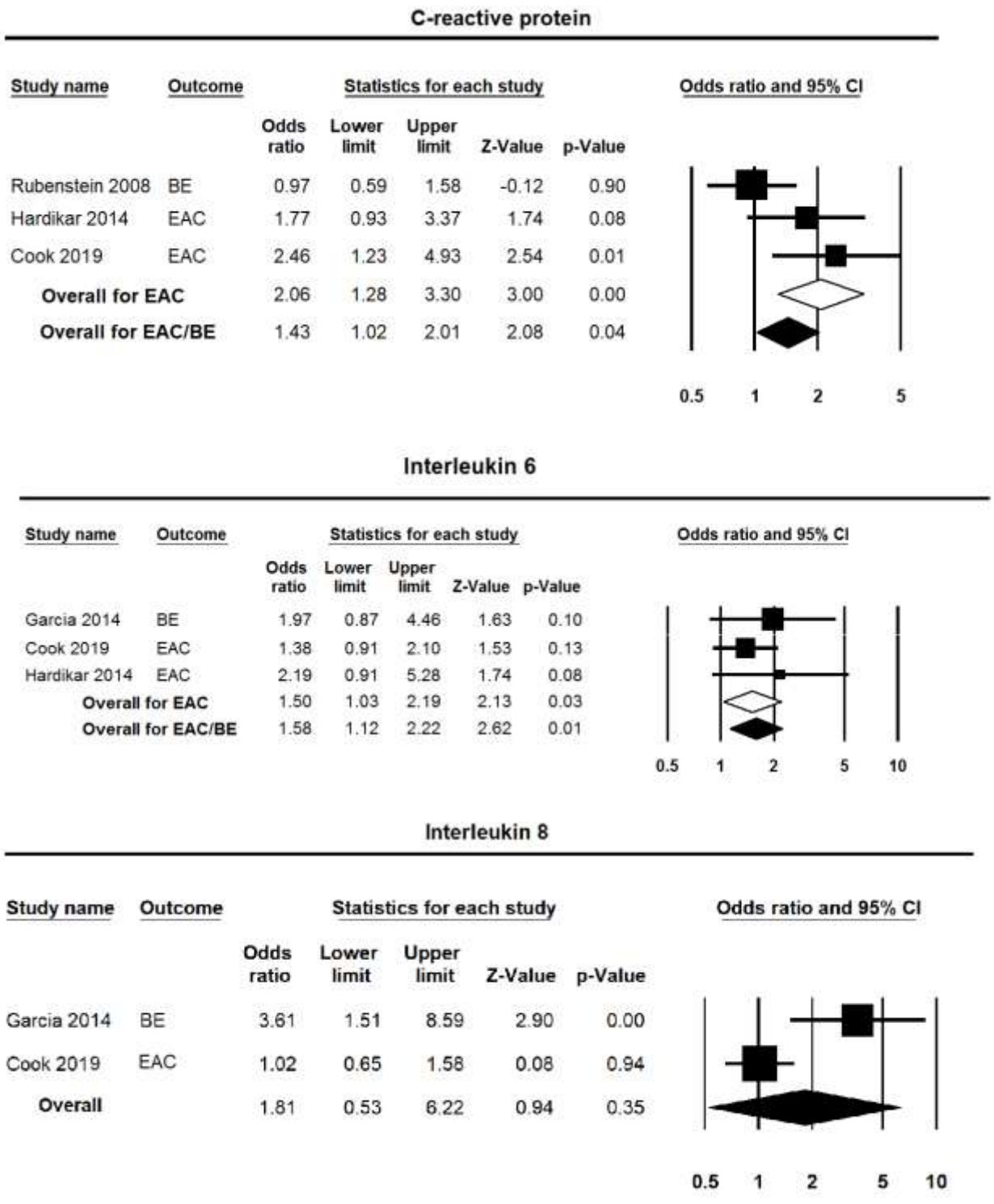
Supplementary Figure S5. Forest plot for the association between circulating glucose levels and risk of esophageal adenocarcinoma. CI: confidence interval.



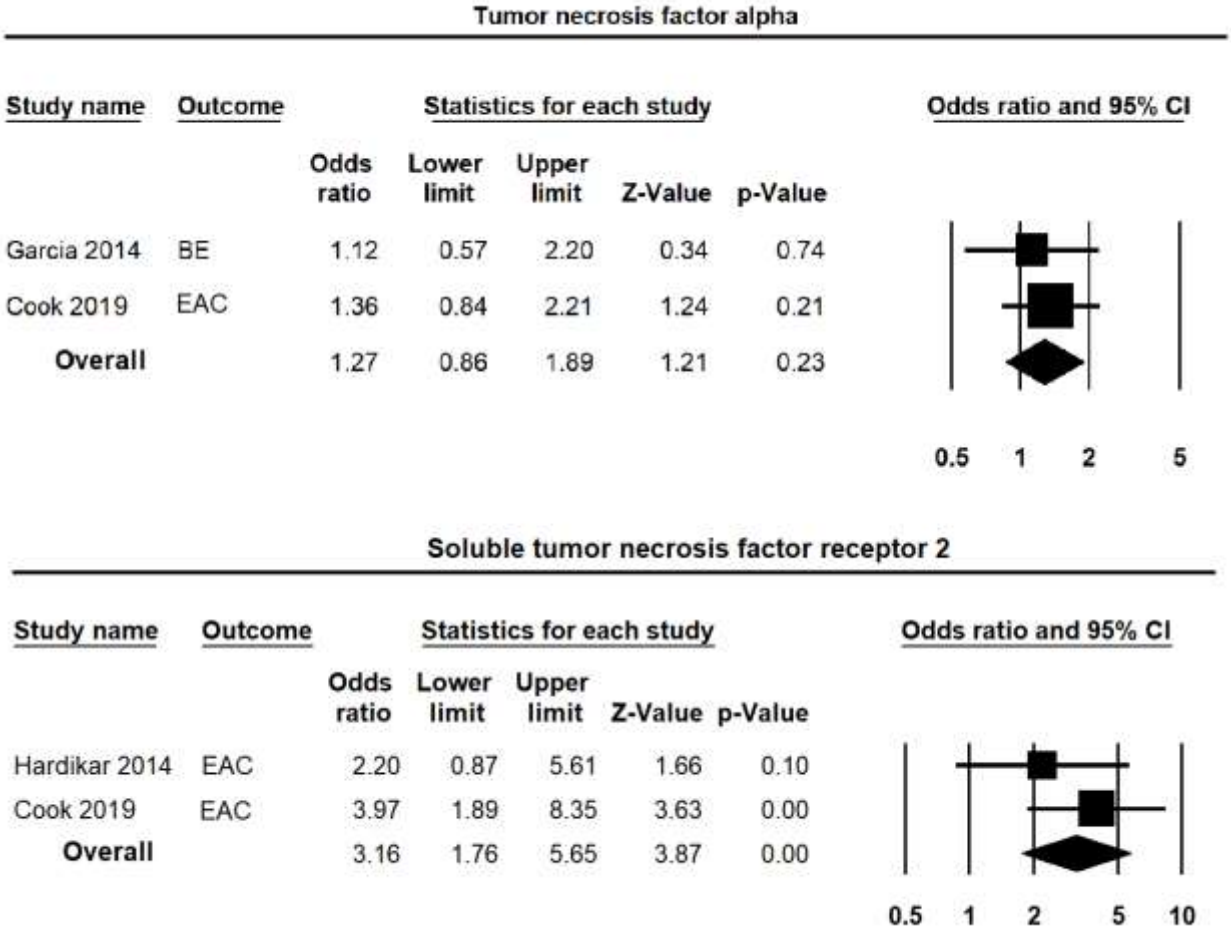
Supplementary Figure S6. Forest plot for the associations between circulating levels of insulin, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 and risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE), comparing the highest versus lowest tertiles. CI: confidence interval.



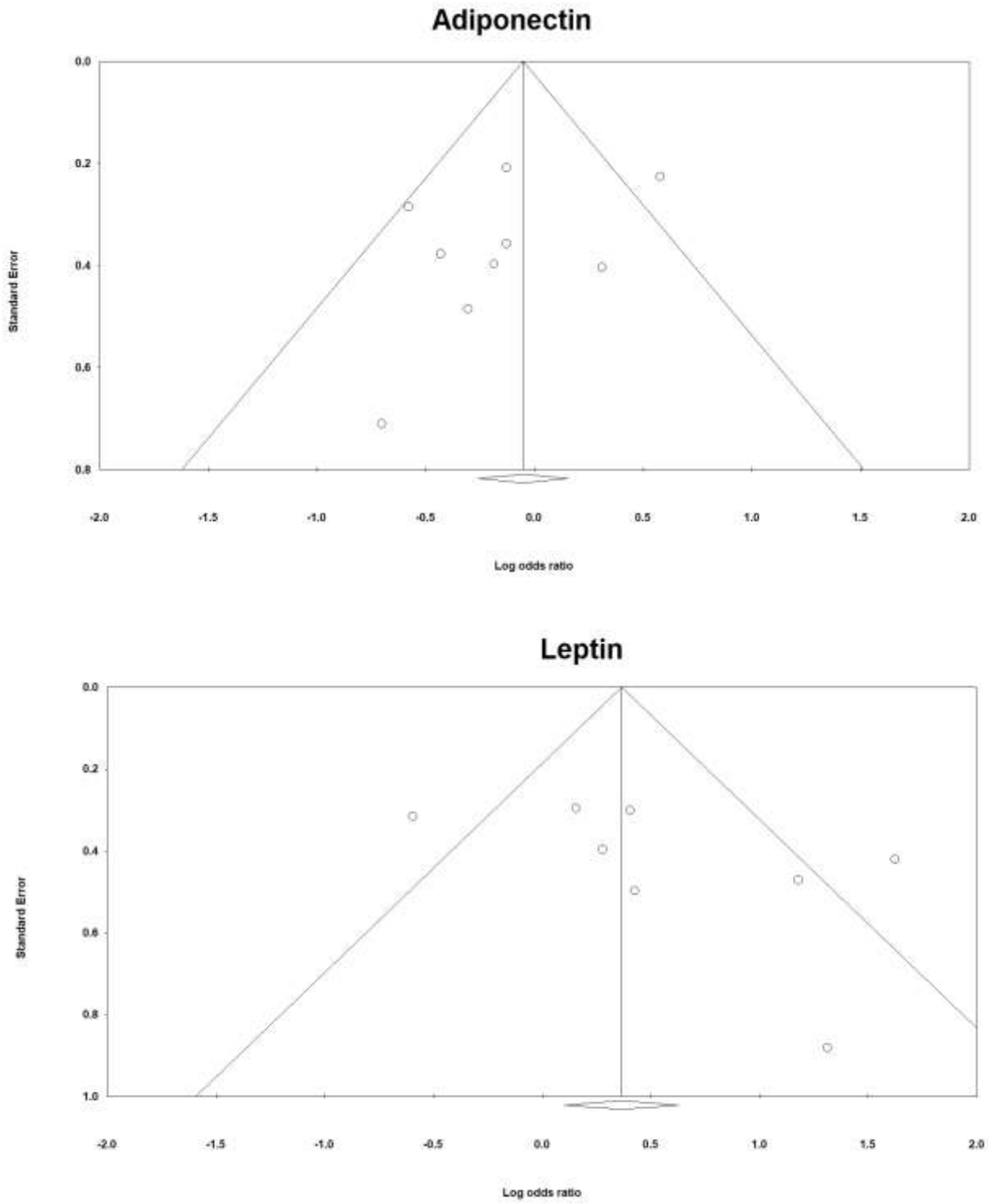
Supplementary Figure S7. Forest plot for the association between elevated circulating triglycerides levels and risk of esophageal adenocarcinoma. CI: confidence interval.



Supplementary Figure S8. Forest plot for the associations of circulating levels of C-reactive protein, interleukin 6 and interleukin 8 with risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE), comparing the highest versus lowest tertiles. CI: confidence interval.



Supplementary Figure S9. Forest plot for the associations of circulating levels tumor necrosis factor alpha and soluble tumor necrosis factor receptor 2 with risk of esophageal adenocarcinoma (EAC) or Barrett's esophagus (BE), comparing the highest versus lowest tertiles. CI: confidence interval.



Supplementary Figure S10. Funnel plots of standard error by log odds ratio for the associations between circulating levels of adiponectin and leptin and risk of esophageal adenocarcinoma or Barrett's esophagus.