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Association of adiponectin multimers with Barrett's oesophagus

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Abstract

Objective—Barrett's oesophagus is associated with abdominal obesity. Adiponectin is a peptide that is secreted from adipocytes and circulates in three multimeric forms: low molecular weight (LMW), middle molecular weight (MMW), and high molecular weight (HMW). The anti-inflammatory effects of adiponectin are specific to individual multimers, with LMW being most anti-inflammatory. We postulated that circulating levels of adiponectin and its multimers would be associated with the risk of Barrett's oesophagus.

Design—Cross-sectional study.

Setting—Outpatient clinic in North Carolina, USA.

Patients—Cases of Barrett's oesophagus and controls undergoing upper endoscopy for gastro-oesophageal reflux disease (GORD).

Main outcome measures—Adjusted odds ratios of plasma adiponectin levels and its multimers for Barrett's oesophagus.

Results—There were 112 cases of Barrett's oesophagus and 199 GORD controls. Total adiponectin was not associated with Barrett's oesophagus (3rd tertile vs 1st tertile adjusted odds ratio (aOR) = 0.88; 95% confidence interval (CI) = 0.44 to 1.78). High levels of LMW adiponectin were associated with a decreased risk of Barrett's oesophagus (3rd tertile vs 1st tertile aOR = 0.33; 95% CI, 0.16 to 0.69), and a high LMW/total ratio appeared particularly inversely associated with Barrett's oesophagus (3rd tertile vs 1st tertile aOR = 0.27; 95% CI, 0.13 to 0.58).

Conclusions—High levels of LMW adiponectin are associated with a decreased risk of Barrett's oesophagus among patients with GORD. Further human studies are required to confirm these findings, and in vitro studies are needed to understand if there is a mechanism whereby adiponectin may affect Barrett's metaplasia.

Oesophageal adenocarcinoma and its accepted precursor, Barrett's oesophagus, are associated with obesity, particularly abdominal obesity.^{1–3} Gastroesophageal reflux disease (GORD) is well described as a risk factor for both Barrett's oesophagus and oesophageal adenocarcinoma.^{4–6} The effect of obesity on the development of Barrett's oesophagus and

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oesophageal adenocarcinoma may be due to an effect of obesity promoting GORD. Possible mechanisms include alterations in the anatomy of the gastro-oesophageal junction and increased abdominal pressure, or confounding by diet.⁷⁻⁹ However, since obesity is a risk factor for other cancers for which there are no known structural mechanisms,¹⁰ at least some of the effect of obesity on the risk for Barrett's oesophagus may be metabolic and mediated by circulating factors related to obesity.¹¹⁻¹³

Adiponectin is a peptide secreted by adipose tissue, whose blood levels are inversely correlated with obesity and lower in men than women.¹⁴ It is involved in regulation of inflammation,^{15,16} and may suppress carcinogenesis by a number of mechanisms.^{17,18} Low circulating levels are associated with an increased risk of cancers of the colon, stomach, prostate, breast, and uterus.¹⁷ Specific receptors for adiponectin (AdipoR1 and AdipoR2) are found in oesophageal mucosa, and adiponectin induces apoptosis in a cell line of oesophageal adenocarcinoma.¹⁹ Adiponectin also inhibits leptin-induced proliferation via AdipoR1 in cell lines of oesophageal adenocarcinoma.²⁰ In a previous small study, we found that low plasma levels of adiponectin are associated with an increased risk of Barrett's oesophagus among patients undergoing upper endoscopy.¹³ However, another pilot study did not detect such a relationship.¹²

Adiponectin circulates in human blood in three multimeric complexes: trimers (low molecular weight, LMW), hexamers (middle molecular weight, MMW), and octadecamers (high molecular weight, HMW).²¹ Most of the actions of adiponectin on insulin resistance and coronary artery disease have been attributed to deficiencies in the circulating levels of the HMW multimer.²²⁻²⁴ In contrast, the three multimers of adiponectin may have contrasting effects on inflammation. The HMW multimer induces the secretion of the pro-inflammatory cytokine interleukin 6 (IL6) from human monocytic cells, but the LMW multimer is anti-inflammatory, suppressing lipopolysaccharide-mediated release of IL6, and stimulating the secretion of the anti-inflammatory cytokine IL10.²⁵

The specific effects of the multimers on the development of cancer have not yet been well studied. Low serum levels of HMW adiponectin are associated with the presence of metastases among normal weight Japanese patients with renal cell carcinoma,²⁶ and with an increased risk for breast cancer.²⁷ The addition of even sub-physiological levels of HMW adiponectin to cell lines of prostate and hepatocellular carcinoma suppresses cell proliferation.²⁸

No prior study has evaluated the effects of the various multimers of adiponectin on the risk of Barrett's oesophagus. Our previously published study in a different patient population was limited to evaluation of total adiponectin levels, and was limited by small sample size and a matching protocol that resulted in difficulty in fully adjusting for gender in the analyses. In the current study, we conducted a separate and larger clinic-based, cross-sectional study of patients with Barrett's oesophagus compared to patients with GORD and no Barrett's oesophagus, and sought to estimate the risk of levels of total adiponectin, and each of its multimers, for Barrett's oesophagus. Since adiponectin levels are inversely associated with insulin levels,^{29,30} and because hyper-insulinaemia might be carcinogenic,^{31,32} we also assessed whether the relationship of adiponectin with Barrett's oesophagus might be confounded by insulin.

METHODS

We performed a clinic-based cross-sectional study comparing levels of adiponectin and its multimers between subjects with reflux symptoms and Barrett's oesophagus and subjects with reflux symptoms but no Barrett's oesophagus. The primary aim of the original study

was to evaluate the relationship between obesity, fat distribution, and Barrett's oesophagus. Blood specimens had been banked for analysis of future hypotheses such as the present study.

Subjects

Subjects were recruited from the gastroenterology clinics of the University of North Carolina between 2002 and 2007, and provided written, informed consent. All enrolled subjects were between the ages of 18 and 80, and had an upper endoscopy planned for investigation of GORD symptoms or for surveillance of known Barrett's oesophagus. Any patient who underwent endoscopy with a primary or secondary indication of reflux disease was eligible to participate in the study. All subjects reported classic GORD symptoms (heartburn, acid regurgitation or waterbrash) to study personnel and had received a physician diagnosis of GORD. Barrett's oesophagus cases included both incident and prevalent diagnoses, and were eligible for inclusion if they had endoscopically evident Barrett's oesophagus of any length. For purposes of this study, Barrett's oesophagus was defined as the presence of both:

- upward displacement of the squamocolumnar junction noted on endoscopy such that the junction of squamous and columnar mucosa is no longer at the interface of the most distal tubular oesophagus and the proximal gastric folds
- intestinal columnar metaplasia, as defined by the presence of columnar epithelium with goblet cells demonstrated on haematoxylin & eosin staining, in at least one biopsy specimen from the tubular oesophagus. In equivocal cases, the documentation of goblet cells by alcian blue staining was considered positive for intestinalised metaplasia.

The finding of intestinal metaplasia in the oesophagus was necessary, but intestinal metaplasia without an endoscopic appearance of Barrett's oesophagus was not sufficient for inclusion as a case of Barrett's oesophagus. All subjects who had endoscopic evidence of Barrett's oesophagus underwent a standardised biopsy protocol, with jumbo or maximum-capacity biopsies in four quadrants every 2 cm throughout the length of the endoscopically abnormal tissue.

Controls for this study consisted of those patients with GORD who did not harbour endoscopically evident Barrett's oesophagus. Controls were recruited using the same protocol as cases.

Exclusion criteria included:

- patients who were unable to read or comprehend the informed consent or written questionnaires
- patients who were status post-partial or complete oesophageal resection
- patients with prevalent Barrett's oesophagus who had undergone endoscopic ablation
- patients found to harbour oesophageal carcinoma on the index endoscopy.

Anthropometry

All body measurements were taken by a trained nurse study coordinator. All subjects were measured in only thin clothing or a gown. All measurements were entered on a standard data entry form. The body measurements were obtained as follows:

- *for hip measurements*: the average of two successive measurements at the largest circumference between the waist and thighs while standing
- *for waist measurements*: the average of two successive measurements at the umbilicus
- *for weight*: the subject's weight, as measured on a balance scale in the clinic
- *for height*: the subject's height in inches.

Assays

Blood was drawn after an overnight fast on the morning of the planned endoscopy. Centrifuge separation of the blood was performed, and samples were aliquoted into 0.1 ml aliquots. Plasma and serum samples were stored frozen until the completion of enrolment, then shipped on dry ice to the University of Michigan. Plasma samples were assayed for adiponectin in the laboratory of one of the investigators (JYK). Available serum samples were assayed for glucose and insulin in the Chemistry Core of the Michigan Diabetes Research and Training Center.

Plasma samples were analysed for adiponectin using a commercially available colorimetric enzyme-linked immunosorbent assay with either no pretreatment, or pretreatment with one of two proteases that specifically digest either the LMW multimer or the LMW and the MMW multimers when compared to quantitative western blot analysis (Alpco Diagnostics, Salem, New Hampshire, USA).³³ This provided results for the levels of total, HMW, and HMW + MMW adiponectin. Each sample was analysed in duplicate. The intra-assay coefficient of variation for each untreated or pretreated assay is less than 6%;³³ however, since the results for LMW, MMW, and ratio of LMW to total adiponectin are derived from the results of total, HMW, and HMW + MMW adiponectin, the coefficient of variation for the derived results are likely greater.

Serum samples were analysed in duplicate for glucose on a Cobas Mira Chemistry Analyzer (Roche Diagnostics, Indianapolis, Indiana, USA), using reagents from Diagnostic Chemicals Limited (Oxford, Connecticut, USA). The intra-assay coefficient of variation is 2% at 84 and 283 mg/dl, and inter-assay coefficient of variation is 2.9% at 82 mg/dl and 2.6% at 278 mg/dl. Serum samples were analysed in duplicate for insulin using a double-antibody radioimmunoassay which utilised a ¹²⁵I-human insulin tracer (Linco Research, St. Charles, Missouri, USA), a guinea pig-anti-porcine insulin first antibody (Michigan Diabetes Research and Training Center, 68.5% cross-reaction to human proinsulin), and a goat-anti-guinea pig gamma globulin-pegylated second antibody (Antibodies, Davis, California, USA) and standardised against the Human Insulin International Reference Preparation (National Institute for Biological Standards and Control). The limit of sensitivity for the assay is 2.1 µU/ml, and inter-assay and intra-assay variabilities are 3.4% and 2.7%, respectively, at 25 µU/ml.

Statistical analysis

Statistical analysis was performed using SAS 9.1. Data were examined for range and logic inconsistencies. The homeostasis model analysis of insulin resistance (HOMA-IR) was calculated from the glucose and insulin levels using the formula:³⁴

$$\text{HOMA-IR} = \text{insulin (mU/l)} \times \text{glucose (mmol/dl)} / 22.5$$

Age, body mass index (BMI), waist circumference, hip circumference, and waist/hip ratio were treated as linear variables after exploring for threshold effects. Subjects were categorised into tertiles of total plasma adiponectin, multimers of adiponectin, serum insulin, serum glucose, and HOMA-IR based on the distribution among control subjects. The associations between continuous variables were examined using Pearson's correlation. Comparisons between groups for continuous variables were performed using the t test. Univariate logistic regression was performed for each variable for the outcome of Barrett's oesophagus, and multivariate stepwise forward logistic regression models were created including adiponectin and each of its multimers. Factors for adjustment were chosen a priori based on the published risk and protective factors for Barrett's oesophagus, as limited by the rule of thumb of requiring 10 outcomes per factor. The factors chosen were age, gender, race, hiatal hernia, and abdominal obesity. Barrett's oesophagus appeared to be more closely associated with waist/hip ratio than the other measures of obesity (waist circumference, hip circumference, BMI), and so was used in the multivariate models to the exclusion of the other measures of obesity to avoid colinearity. Effect modification was deemed present if odds ratios stratified by a categorical variable differed by more than 10%, and was tested for statistical significance by the Wald χ^2 for a cross-product term introduced into the unstratified logistic regression model. The effects of waist/hip ratio, hiatal hernia, adiponectin and its multimers appeared to be modified by gender, but did not reach statistical significance. Since the differences in these stratified and unstratified odds ratios were greater than the a priori threshold of 10%, but the differences did not reach statistical significance, models are presented both unstratified by gender (adjusting for age, gender, waist/hip ratio, hiatal hernia, and race) and stratified by gender (adjusting for the same factors except race due to smaller numbers in the strata). Since adiponectin levels are inversely associated with serum insulin levels, and hyperinsulinemia might have direct effects on carcinogenesis, the possibility of confounding was assessed by further adjusting the models for insulin levels or HOMA-IR.

RESULTS

Baseline characteristics

A total of 536 subjects were potentially eligible for this study and all of these were screened for eligibility. Of subjects with Barrett's oesophagus, 137 were found to be eligible for the study, and 112 (82%) participated. Of control subjects, 280 subjects were eligible for the study, and 199 subjects participated (71%). The most common reason for declining participation was unwillingness to undergo an additional phlebotomy. Because of the cross-sectional nature of the study, all subjects consenting to participation completed the study.

The baseline characteristics of the subjects are presented in table 1. Patients with Barrett's oesophagus were older, and there was a higher proportion of men and whites than GORD controls. There was no difference in BMI between groups, but cases had larger average waist circumference and smaller hip circumference. Cases included a higher proportion with hiatal hernia. There was no difference in total plasma adiponectin (sum of all multimers), but cases of Barrett's oesophagus had higher mean HMW adiponectin, and lower mean MMW and LMW adiponectin. The average ratio of LMW to total adiponectin was lower in Barrett's oesophagus cases than in GORD controls. The distributions of the LMW levels and of the LMW to total adiponectin ratios are displayed stratified by case/control status and gender in fig 1 and 2.

Among controls, plasma levels of total and LMW adiponectin were weakly and inversely correlated with BMI in both men ($\rho = -0.27$, $p = 0.03$, and $\rho = -0.11$, $p = 0.37$ for total and LMW, respectively) and women ($\rho = -0.37$, $p < 0.0001$, and $\rho = -0.22$, $p = 0.01$ for total and LMW, respectively), and inversely correlated with waist/hip ratio among women

only ($\rho = -0.24$, $p = 0.01$, and $\rho = -0.10$, $p = 0.26$ for total and LMW, respectively). Similar gender-specific inverse correlations were found among cases, between HMW or MMW adiponectin and BMI or waist/hip ratio, and between multimers and waist circumference. The ratio of LMW to total adiponectin was not correlated with either BMI or waist/hip ratio among controls or among male cases, but was moderately correlated with BMI ($\rho = 0.41$, $p = 0.01$) and waist/hip ratio ($\rho = 0.51$, $p = 0.001$) among female cases. Female control subjects had higher total levels of adiponectin than male controls ($6.39 \mu\text{g/ml}$ vs 5.13 , $p = 0.002$), and higher levels of HMW ($2.68 \mu\text{g/ml}$ vs 2.00 , $p = 0.007$), and MMW forms ($1.45 \mu\text{g/ml}$ vs 1.13 , $p = 0.02$), but no difference was detected in levels of LMW ($2.26 \mu\text{g/ml}$ vs 2.01 , $p = 0.11$) or the ratio of LMW to total adiponectin (0.38 vs 0.41 , $p = 0.19$). Adiponectin levels in non-obese GORD controls are presented in table 2.

Association between adiponectin and Barrett's oesophagus

The total plasma level of adiponectin was not associated with the risk of Barrett's oesophagus compared to GORD controls (adjusted odds ratio (aOR) 3rd tertile vs 1st tertile, aOR = 0.88; 95% CI, 0.44 to 1.78) (table 3). However, the highest tertile of LMW adiponectin was associated with one-third the risk of Barrett's oesophagus, adjusting for waist/hip ratio, gender, hiatal hernia, age, and race (3rd vs 1st tertiles aOR = 0.33; 95% CI, 0.16 to 0.69). The ratio of LMW to total adiponectin was most closely inversely associated with the risk of Barrett's oesophagus (3rd vs 1st tertiles aOR = 0.27; 95% CI, 0.13 to 0.58). In contrast, higher levels of HMW adiponectin were marginally associated with an increased risk of Barrett's oesophagus (3rd vs 1st tertiles aOR = 1.96; 95% CI, 0.96 to 4.03). Full logistic regression models adjusting for waist circumference rather than waist/hip resulted in similar point estimates (data not shown).

Analyses stratified by gender

The effect of a number of variables (waist/hip ratio, hiatal hernia, adiponectin and its multimers) appeared to be modified by gender; therefore, analyses were also performed stratifying by gender (table 4). Although subject numbers in some cells are small, this analysis revealed a possibly stronger association of low levels of LMW adiponectin with Barrett's oesophagus among women than among men. Although the adjusted odds ratio was less than 0.5 among men, the association between LMW adiponectin levels and Barrett's oesophagus lost statistical significance, perhaps due to small sample size in the stratified analyses. However, the association of low LMW/total adiponectin ratio with Barrett's oesophagus remained strong and statistically significant among both genders. This association also appeared stronger among women than among men.

Because adiponectin levels are affected by menopause,²⁹ we performed analyses in 107 of the 169 women in whom menstrual status was known. Adjusting for age, post-menopausal status was not associated with Barrett's oesophagus (OR = 1.82; 95% CI, 0.37 to 8.90). There were only three pre-menopausal women with Barrett's oesophagus, limiting the power of the analysis. Nonetheless, the association of adiponectin multimers with Barrett's oesophagus among women appeared similar after adjustment for menopausal status in addition to adjustments for age, hiatal hernia, and waist/hip ratio (LMW 3rd tertile vs 1st tertile of aOR = 0.20; 95% CI, 0.04 to 0.99; LMW/Total 3rd tertile vs 1st tertile of aOR = 0.33; 95% CI, 0.07 to 1.5).

Assessment for confounding by hyperinsulinaemia

Among controls, plasma levels of total and LMW adiponectin were weakly and inversely correlated with serum levels of insulin ($\rho = -0.31$, $p < 0.0001$, and $\rho = -0.20$, $p = 0.006$ for total and LMW, respectively), and with the HOMA-IR ($\rho = -0.21$, $p = 0.005$, and $\rho = -0.16$, $p = 0.04$ for total and LMW, respectively), but not with serum glucose levels ($\rho =$

–0.09, $p = 0.24$, and $\rho = -0.05$, $p = 0.52$ for total and LMW, respectively). However, the ratio of LMW to total adiponectin was not correlated with insulin ($\rho = 0.12$, $p = 0.12$) or with HOMA-IR ($\rho = 0.05$, $p = 0.53$). Similar correlations were observed across genders. Among cases of Barrett's oesophagus, there appeared to be closer inverse correlation between total adiponectin with HOMA-IR ($\rho = -0.29$, $p = 0.0037$), and also a weakly positive correlation between the ratio of LMW to total adiponectin with insulin ($\rho = 0.20$, $p = 0.044$) and with HOMA-IR ($\rho = 0.17$, $p = 0.08$).

Serum insulin levels were not associated with Barrett's oesophagus (3rd tertile vs 1st tertile aOR = 0.87; 95% CI, 0.41 to 1.8), adjusting for gender, age, hiatal hernia, race, and waist/hip ratio. Neither were serum glucose levels (3rd tertile vs 1st tertile aOR = 1.0; 95% CI, 0.49 to 2.1) or HOMA-IR (3rd tertile vs 1st tertile aOR = 0.63; 95% CI, 0.29 to 1.3).

Multivariate logistic regression was repeated, adjusting for insulin in addition to gender, age, hiatal hernia, race, and waist/hip ratio. Higher levels of LMW adiponectin remained inversely associated with Barrett's oesophagus in this model (3rd tertile vs 1st tertile aOR = 0.35; 95% CI, 0.16 to 0.77). Adjusting for HOMA-IR rather than insulin resulted in nearly identical estimates (3rd tertile vs 1st tertile aOR = 0.35; 95% CI, 0.16 to 0.76). Likewise, the ratio of LMW to total adiponectin remained inversely associated with Barrett's oesophagus in models further adjusted for insulin (3rd tertile vs 1st tertile aOR = 0.20; 95% CI, 0.084 to 0.46) or HOMA-IR (3rd tertile vs 1st tertile aOR = 0.21; 95% CI, 0.092 to 0.49).

DISCUSSION

We performed a clinic-based study estimating the association between circulating levels of multimers of adiponectin and the presence of Barrett's oesophagus. We did not find any association between the total level of adiponectin and the presence of Barrett's oesophagus, but did find that higher levels of the LMW multimers (trimers) of adiponectin were associated with one-third the risk of Barrett's oesophagus. These effects may be stronger among women than among men, but we did not find a statistically significant interaction between adiponectin multimers and gender.

If adiponectin levels do influence the risk of Barrett's oesophagus, what might be the mechanism? Adiponectin can bind to growth factors, thereby inhibiting the growth factors' interaction with their cell membrane receptors.³⁵ Adiponectin activates the 5'-AMP-activated protein kinase (AMPK) pathway, thereby suppressing cell proliferation, perhaps in part by regulating p21 and p53.¹⁸ Adiponectin also suppresses expression of cyclin D1, possibly via regulation of the β -catenin–Wnt pathway.¹⁸ Cyclin D1, Wnt and p53 are involved in neoplastic progression in Barrett's oesophagus.^{36–38} Indeed, adiponectin induces apoptosis in a cell line of oesophageal adenocarcinoma,¹⁹ and inhibits leptin-induced proliferation in cell lines of oesophageal adenocarcinoma.²⁰ However, most of these studies have not attempted to distinguish the effects of the various multimers, and the effect of adiponectin on the metaplastic event has not been directly studied in vitro. Since Barrett's metaplasia is believed to be an aberrant response in the setting of erosive oesophagitis,³⁹ an attractive hypothesis is that normal levels of circulating LMW adiponectin are sufficient to suppress the inflammatory response to GORD,²⁵ or guide the healing response toward regeneration of squamous mucosa. For instance, LMW adiponectin might suppress the local expression of IL6 in oesophageal mucosa.²⁵ IL6 expression has been shown to be increased in the epithelium of Barrett's oesophagus.⁴⁰ In the setting of low levels of LMW adiponectin, the response to GORD might be directed toward a more exuberant oesophagitis and/or metaplasia into intestinal epithelium. Our finding that a low ratio of LMW to total adiponectin may be particularly associated with Barrett's oesophagus could be explained by the opposing effects of different multimers on inflammation.²⁵ Although high LMW or high

LMW to total adiponectin ratio appeared most strongly protective of Barrett's oesophagus in the current study, a positive but weaker association was observed between high levels of HMW adiponectin and Barrett's oesophagus. It is not clear whether any effect of adiponectin on Barrett's oesophagus is mediated by protection by LMW adiponectin, promotion by HMW adiponectin or both.

Adiponectin is one of many circulating factors associated with obesity, and the observed effect of LMW adiponectin may be confounded by any or many of these factors that were not measured in this study. For instance, IL6 downregulates adipocyte expression of adiponectin,⁴¹ and circulating IL6 or other cytokines might instead be responsible for Barrett's metaplasia. Total adiponectin is inversely associated with insulin, and hyperinsulinaemia could instead be the causative factor related to Barrett's oesophagus.²⁹³⁰ However, the insulin-sensitising effect of adiponectin appears to be specifically due to the HMW multimer.²² We found no association between serum insulin levels, or between an estimate of insulin resistance, and the presence of Barrett's oesophagus. Further adjusting for insulin levels or insulin resistance did not appreciably alter the observed association between LMW adiponectin and Barrett's oesophagus. Menopause is associated with an increase in total adiponectin,²⁹ and studies of the association of adiponectin with breast cancer risk have generally found an inverse association only among post-menopausal women.⁴²⁻⁴⁴ The stronger effect of LMW adiponectin on Barrett's oesophagus that we observed among women might be due to interactions with sex hormones, or an epiphenomenon. Unfortunately, we had too few pre-menopausal women with Barrett's oesophagus (n = 3) to draw any conclusions regarding an interaction between menopausal status and adiponectin multimers for the risk of Barrett's oesophagus.

The results of this study differed from our pilot study in a different population finding evidence for an association between Barrett's oesophagus and low levels of total adiponectin.¹³ This may have been due to differences in subject populations or the assay used to measure adiponectin. Also, the association in that study with total adiponectin became statistically insignificant after adjusting for waist/hip ratio. Our current null finding is consistent with a small pilot study performed by another group.¹² However, unlike the present work, neither of these previous studies measured levels of the specific multimers. Although the present study involved more than 300 subjects, certain stratified analyses were limited by small numbers. The present study is limited by the potential for unmeasured confounders such as dietary habits. A diet high in whole grain cereals is positively correlated with circulating levels of total adiponectin, but to our knowledge dietary habits have not been correlated with circulating levels of the adiponectin multimers.⁴⁵ Other potential unmeasured confounders include physical activity, *Helicobacter pylori* infection, and the use of non-steroidal anti-inflammatory drugs. Despite our attempts to control for multiple factors, there may have also been residual confounding by obesity or GORD. The present study did not have access to adiponectin levels in a control group without GORD; levels of adiponectin and its multimers in our GORD control group were higher than those found in non-obese men without insulin resistance, but these differences may be due to other differences in study populations or assays.²⁵ Comparing adiponectin levels to a non-obese population without GORD and without Barrett's oesophagus could yield further understanding of the mechanism of the effect of adiponectin on Barrett's oesophagus. Another limitation is the inclusion of both incident and prevalent cases of Barrett's oesophagus, and the clinic-based nature of the study. While a population-based design would be preferable, the costs associated with recruiting subjects with GORD, then identifying over 100 Barrett's oesophagus cases from this population were prohibitive. The strengths of our study include the rigorous data collection by trained research assistants, the prospective nature of our data collection and specimen banking, the low likelihood of

misclassification due to the rigorous biopsy protocol, and the measurement of body anthropomorphic measurements on-site by trained study staff.

In summary, we found a strong inverse relationship between circulating levels of LMW adiponectin and the presence of Barrett's oesophagus among patients with GORD. If noted in other studies, this finding has implications both for models of pathogenesis of Barrett's oesophagus, as well as for potential use as a biomarker of disease. Further human studies are required to confirm our findings, and in vitro studies are needed to understand if there is a mechanism whereby adiponectin may promote Barrett's metaplasia.

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Figure 1. Box-plot of low molecular weight adiponectin. Each box-plot displays the 10th, 25th, 50th, 75th, and 90th percentiles of low molecular weight (LMW) adiponectin in cases of Barrett's oesophagus and GORD controls, stratified by gender.

Figure 2.

Box-plot of ratio of low molecular weight to total adiponectin. Each box-plot displays the 10th, 25th, 50th, 75th, and 90th percentiles of the ratio of low molecular weight (LMW) to total adiponectin in cases of Barrett's oesophagus and GORD controls, stratified by gender.

Table 1

Baseline characteristics

	<u>Barrett's oesophagus cases</u>	<u>GORD controls</u>	
	Mean (SD) or number (%)	Mean (SD) or number (%)	p Value
Number of subjects	112	199	
Age (years)	57.4 (11.3)	50.7 (13.3)	<0.0001
Gender			<0.0001
Male	73 (65)	69 (35)	
Female	39 (35)	130 (65)	
Race			0.003
White	106 (95)	160 (80)	
Black	4 (4)	30 (15)	
Other	2 (2)	9 (5)	
Smoking status (ever smokers)	53 (50)	95 (48)	0.68
BMI (kg/m ²)	28.2 (5.1)	28.7 (6.2)	0.46
Waist circumference (cm)	94.3 (15.2)	91.6 (16.3)	0.18
Hip circumference (cm)	101.7 (12.7)	104.2 (14.7)	0.15
Waist/hip ratio	0.93 (0.10)	0.88 (0.09)	<0.0001
Hiatal hernia	60 (57)	74 (38)	0.002
Adiponectin			
Total (µg/ml)	6.03 (3.32)	5.95 (2.87)	0.83
High molecular weight (µg/ml)	3.09 (2.41)	2.44 (1.81)	0.01
Middle molecular weight (µg/ml)	1.13 (0.82)	1.34 (1.08)	0.06
Low molecular weight (µg/ml)	1.80 (1.09)	2.17 (1.08)	0.004
Low molecular weight/total ratio	0.33 (0.13)	0.39 (0.16)	0.0002

Results are given as the mean (SD) or number (%).

GORD, gastro-oesophageal reflux disease.

Table 2

Plasma adiponectin in non-obese GORD controls

	Males mean (SD)	Females mean (SD)	p Value
Number of subjects	47	59	
Total (μg/ml)	5.31 (2.43)	6.32 (2.25)	0.03
High molecular weight (μg/ml)	2.14 (1.63)	2.51 (1.38)	0.21
Middle molecular weight (μg/ml)	1.11 (0.73)	1.42 (0.83)	0.05
Low molecular weight (μg/ml)	2.06 (0.95)	2.39 (1.12)	0.10
Low molecular weight/total ratio	0.41 (0.15)	0.39 (0.15)	0.63

Table 3

Plasma adiponectin and Barrett's oesophagus

		OR (95% CI) [*]	OR (95% CI) [†]
No. of Barrett's oesophagus/no. of GORD		OR (95% CI) [*]	Adjusted for waist/hip ratio
Total adiponectin			
1 st tertile	44/67	1.00 (reference)	1.00 (reference)
2 nd tertile	32/66	0.59 (0.30 to 1.15)	0.65 (0.32 to 1.30)
3 rd tertile	36/66	0.87 (0.45 to 1.66)	0.88 (0.44 to 1.78)
HMW adiponectin			
1 st tertile	29/67	1.00 (reference)	1.00 (reference)
2 nd tertile	35/66	1.13 (0.58 to 2.23)	1.02 (0.50 to 2.08)
3 rd tertile	48/66	1.81 (0.92 to 3.53)	1.96 (0.96 to 4.03)
MMW adiponectin			
1 st tertile	42/67	1.00 (reference)	1.00 (reference)
2 nd tertile	43/66	0.95 (0.51 to 1.77)	0.96 (0.50 to 1.85)
3 rd tertile	27/66	0.73 (0.370 to 1.43)	0.82 (0.40 to 1.68)
LMW adiponectin			
1 st tertile	52/67	1.00 (reference)	1.00 (reference)
2 nd tertile	41/66	0.88 (0.48 to 1.60)	0.84 (0.45 to 1.58)
3 rd tertile	19/66	0.36 (0.18 to 0.72)	0.33 (0.16 to 0.69)
LMW/total			
1 st tertile	54/66	1.00 (reference)	1.00 (reference)
2 nd tertile	42/66	0.63 (0.35 to 1.16)	0.61 (0.32 to 1.15)
3 rd tertile	16/67	0.30 (0.15 to 0.62)	0.27 (0.13 to 0.58)

^{*} Odds ratios are adjusted for gender, hiatal hernia, age and race.

[†] Odds ratios are adjusted for waist/hip ratio, gender, hiatal hernia, age and race.

CI, confidence interval; GORD, gastro-oesophageal reflux disease; OR, odds ratio.

Table 4

Plasma adiponectin and Barrett's oesophagus, stratified by gender

Men			Women		
	No. of Barrett's oesophagus/no. of GORD (n = 73/69)	OR (95% CI)		No. of Barrett's oesophagus/no. of GORD (n = 39/130)	OR (95% CI)
Total adiponectin					
1 st tertile	34/33	1.00 (reference)		11/44	1.00 (reference)
2 nd tertile	21/22	0.72 (0.29 to 1.79)		10/34	0.60 (0.20 to 1.84)
3 rd tertile	18/14	0.93 (0.35 to 2.48)		18/52	0.90 (0.32 to 2.52)
HMW adiponectin					
1 st tertile	22/32	1.00 (reference)		7/35	1.00 (reference)
2 nd tertile	27/21	1.64 (0.68 to 3.96)		8/45	0.47 (0.14 to 1.67)
3 rd tertile	24/16	1.72 (0.65 to 4.53)		24/50	1.81 (0.63 to 5.16)
MMW adiponectin					
1 st tertile	33/28	1.00 (reference)		9/39	1.00 (reference)
2 nd tertile	27/24	0.87 (0.37 to 2.03)		16/42	1.26 (0.45 to 3.52)
3 rd tertile	13/17	0.65 (0.23 to 1.82)		14/49	1.12 (0.40 to 3.18)
LMW adiponectin					
1 st tertile	37/25	1.00 (reference)		15/42	1.00 (reference)
2 nd tertile	23/23	0.85 (0.35 to 2.09)		18/43	0.76 (0.30 to 1.92)
3 rd tertile	13/21	0.44 (0.17 to 1.16)		6/45	0.23 (0.07 to 0.77)
LMW/total					
1 st tertile	31/18	1.00 (reference)		23/48	1.00 (reference)
2 nd tertile	30/27	0.64 (0.26 to 1.55)		12/39	0.59 (0.24 to 1.48)
3 rd tertile	12/24	0.32 (0.12 to 0.88)		4/43	0.20 (0.06 to 0.69)

Odds ratios are adjusted for waist/hip ratio, hiatal hernia, and age.

GORD, gastro-oesophageal reflux disease; HMW, high molecular weight (adiponectin); CI, confidence interval; LMW, low molecular weight; MMW, middle molecular weight; OR, odds ratio.