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Association Between Circulating Levels of Sex Steroid Hormones and Barrett's Esophagus in Men: a Case–Control Analysis

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Abstract

Background & Aims—Esophageal adenocarcinoma is believed to result from the progression of gastroesophageal reflux disease to erosive esophagitis and re-epithelialization of the esophagus with a columnar cell population termed Barrett's esophagus (BE). Men develop BE and esophageal adenocarcinoma more frequently than women, and the ratio is increasing; approximately 7 men are diagnosed with malignancy for every woman, yet little is known about the mechanisms of this difference. We assessed whether sex steroid hormones were associated with BE in a male population.

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Methods—We analyzed data from the Barrett's Esophagus Early Detection Case Control Study, based at the Walter Reed National Military Medical Center. Blood samples were collected from 173 men with BE and 213 men without BE (controls, based on endoscopic analysis); 13 sex steroid hormones were measured by mass spectrometry and sex hormone binding globulin was measured by ELISA. We also calculated free estradiol, free testosterone and free dihydrotestosterone (DHT). We used multivariable logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age, race, smoking status, alcohol consumption, body mass index (BMI; kg/m^2), heartburn, regurgitation, and gastroesophageal symptom score (excluding heartburn and regurgitation).

Results—Levels of free testosterone and free DHT were positively associated with BE risk; patients in the highest quartile for these hormones were most likely to have BE (for free testosterone, OR=5.36; 95% CI, 2.21–13.03; *P*=0.0002 and for free DHT, OR=4.25, 95% CI, 1.87–9.66; *P*=.001). Level of estrone sulfate was inversely associated with BE risk (*P* for trend=.02). No other hormone was associated with BE risk. Relationships were not modified by age or BMI.

Conclusions—In an analysis of men, levels of free testosterone and free DHT were significantly associated with risk of BE.

Keywords

BEEDS; SHBG; gonadal steroid hormones; esophageal neoplasms; cancer risk

Introduction

The extraordinary and progressively widening sex ratio observed during the natural history of erosive esophagitis to Barrett's esophagus (BE) to esophageal adenocarcinoma is dramatic, peaking at malignancy with more than seven males diagnosed for every female [1, 2]. Analyses of Barrett's and Esophageal Adenocarcinoma CONsortium (BEACON) data [3-8] and other population-based studies [9-13] have enabled assessment of how risk factors may differ between the sexes, yet no analysis to-date has been able to explain the large sex disparities of this disease.

Gastroesophageal reflux is one of the primary risk factors for development of BE and esophageal adenocarcinoma, yet reflux symptoms are approximately equal between the sexes [2, 9] and associations of reflux with esophageal adenocarcinoma are equal or stronger for females compared with males [8]. Body mass index (BMI) is another major risk factor for esophageal adenocarcinoma [5] and an analysis of BE has shown that abdominal obesity may be of greatest importance [6]; these relationships do not appear to be altered or attenuated when adjusted for, or stratified by, symptomatic gastroesophageal reflux. Moreover, the strengths of these associations are similar in both sexes, and population attributable risks estimable from published associations between waist circumference and BE (females=55%, males=37%) [6] and BMI and esophageal adenocarcinoma (females=28%, males=33%) [5] are approximately equal between the sexes or stronger for females compared with males. This evidence, as well as postulated mechanisms of association between obesity and other inflammation-related cancers, have led to the proposition that systemic inflammation may

partly account for the strong relationship between obesity and the pathogenesis of esophageal adenocarcinoma. The evidence base that sex steroid hormones are involved in inflammatory processes [14-17] and the fact that sex steroid hormone receptor proteins are expressed in esophageal tissues [18, 19] supports the hypothesis that sex steroid hormones may underlie sex disparities in the pathogenesis of BE and esophageal adenocarcinoma [20]. We assessed this hypothesis by conducting the first analysis of circulating sex steroid hormones in relation to BE.

Methodology

Study Population

BE cases and endoscopy controls macroscopically-negative for BE were recruited between 2004 and 2012 as part of the Barrett's Esophagus Early Detection Case Control Study (BEEDS) which was based at the Walter Reed National Military Medical Center (WRNMMC) in Bethesda, MD. BE cases were required to have histologic confirmation of specialized intestinal metaplasia with goblet cells; prevalent and incidence cases were eligible. Endoscopy control patients were referred for endoscopy for a variety of reasons including dyspepsia, reflux symptoms, and anemia and were frequency-matched to BE patients on sex. Individuals had to be at least 18 years of age to be eligible for inclusion and were excluded if they had severe pulmonary or cardiac disease, were pregnant, had an inability to give consent, had an active malignancy or were diagnosed with such in the past 5 years (excluding non-melanoma skin cancer). Participants provided a 15 mL blood sample, were interviewed for information on demographics, BMI, and lifestyle factors—including a modified version of a 7-question “GERD Questionnaire” [21]—and had clinical data abstracted from medical records. For this analysis, selection was restricted to males because there were too few females to provide adequate statistical power for a female-only analysis. We selected all males that had ≥0.6 ml of serum for analysis, which resulted in 212 controls and 173 BE cases providing 80% power to detect an odds ratio (OR) of 1.8 based on a median split at alpha 0.05. BEEDS was approved by the NCI Clinical Center IRB and the National Naval Medical Center IRB.

Laboratory analysis

In collaboration with the Pharmacogenomics Laboratory of Laval University, Quebec, Canada, we quantitatively assessed: dehydroepiandrosterone (DHEA), androstenediol, androstenedione, testosterone, dihydrotestosterone (DHT), androsterone (ADT), estrone (E1) and estradiol (E2) using gas chromatography–mass spectrometry (GC-MS); dehydroepiandrosterone-sulphate (DHEAS), 3-androstanediol-3 glucuronide (3α-diol-3G), 3-androstanediol-17 glucuronide (3α-diol-17G), androsterone glucuronide (ADT-G), and estrone sulfate (E1S) using liquid chromatography tandem mass spectrometry (LC-MS/MS); and sex hormone binding globulin (SHBG) using ELISA (Diagnostics Biochem Canada, Inc.). These selected sex steroid hormones cover a wide array and key positions of the sex steroid biosynthesis pathway (Figure). We included 5% quality control (QC) samples from three healthy male individuals, aged 24, 39, and 46 years at blood draw. All coefficients of variation (CVs) were <15% (mean 8%, standard deviation 3%) except DHEAS (17%), E1S

(18%), and ADT (19%). The CV for SHBG quantified by ELISA was 23% which is within manufacturer's expected range of <25%.

Statistical Analysis

We used unconditional logistic regression models to estimate ORs and 95 percent confidence intervals (95%CI). Each exposure was assessed as quartiles using cut-points based on the control distribution, as well as a continuous metric with standardization to half the value of the interquartile range. In addition to assessing individual exposures, we also assessed *a priori* specified combinations and ratios of hormones that are near each other in the metabolic pathway: parent estrogens (the sum of E1 and E2), testosterone:parent estrogens ratio, testosterone:E2 ratio, and androstenedione:E1 ratio. We calculated free estradiol [22], free testosterone [23], and free DHT [24] using formulas that include the individual hormone, SHBG and a constant for albumin.

Minimally adjusted models included age (quartiles) as a covariate. We also assessed whether adjustment for race (white/non-white or unknown) smoking status (ever/never), pack-years of smoking (tertiles), alcohol consumption (never/monthly/weekly/daily), BMI (kg/m^2), heartburn (never/monthly/weekly/daily), regurgitation (never/monthly/weekly/daily), gastroesophageal symptom score (excluding heartburn and regurgitation) and separate groupings of current medications (proton pump inhibitors, H_2 receptor antagonists, antacids, non-steroidal anti-inflammatory drugs) consistently changed OR estimates by more than 10%. None of these covariates consistently affected ORs, but we included age, race, smoking status, alcohol consumption, BMI, heartburn, regurgitation, and gastroesophageal symptom score in the fully-adjusted model given previous evidence that these exposures are associated with BE. We also assessed whether relationships between exposures and BE were modified by age or BMI by conducting stratified analyses based on the median control values. All tests were two-sided and p-values <0.05 were considered to be statistically significant. Analyses were conducted using STATA version 11 (Stata-Corp LP, College Station, TX).

Results

There were 212 controls and 173 BE cases for analysis (Table 1). Cases were more likely to be older, to have ever-smoked, and to have consumed alcohol daily.

Partial correlation coefficients of hormones and SHBG adjusted for age (continuous) and BMI (continuous) amongst control subjects (Supplemental Table 1) provided strongest coefficients for the pairings of free estradiol and E2 (r=0.92), free E2 and E1 (r=0.75), free testosterone and testosterone (r=0.75), estradiol and estrone (r=0.74), and free DHT and DHY (r=0.74).

Table 2 shows the results of the multivariable analyses of all quantitated exposures. Testosterone did share a positive association with BE, with the fourth quartile compared with the first providing an OR of 2.26 (95% CI: 1.03, 4.95, p=0.04), although the test for trend was not statistically significant (p=0.29). High levels of E1S were significantly

inversely associated with BE, as shown by the OR for the continuous analysis (0.59, 95%CI: 0.38, 0.92, p=0.02).

Table 3 shows the results of combinations and ratios as well as the calculated free hormones. Consistent with the testosterone results shown in Table 2, increasing free testosterone was also associated with BE. This association was particularly strong and progressively increased with each subsequent quartile (p for trend=0.003), peaking in the highest with an OR of 5.36 (95%CI: 2.21, 13.03, p=0.0002). Although the testosterone:parent estrogens ratio also provided results supportive of an effect for testosterone, there was no obvious trend in the quartile estimates and additional adjustment for free testosterone attenuated the estimates (data not shown) indicating that the effect was mediated by free testosterone. Free DHT was similarly positively associated with BE with an OR of 4.25 (95% CI: 1.87, 9.66) for the fourth quartile compared with the first. None of the other hormone metrics shown in Tables 2 and 3 appeared to share a relationship with BE.

There was little evidence for any effect modification by age (Supplemental Table 2) or by BMI (Supplemental Table 3) in the stratified analyses.

Discussion

In this analysis of serum sex steroid hormones in relation to BE in men, we found evidence for strong positive associations with free testosterone and with free DHT. In addition, we also observed an inverse association with high levels of E1S. There was no evidence that these relationships were modified by age or BMI.

The large sex disparities of BE [2] and esophageal adenocarcinoma [1], coupled with strong relationships with obesity [5, 6], have led to hypotheses that sex steroid hormones may underlie these observations [13, 20, 25-27]. Hypotheses include a protective role for estrogens; a carcinogenic effect of androgens; and/or effects caused by alterations in the ratio of androgens to estrogens. While no previous study has assessed circulating sex steroid hormones in relation to BE, one previous small case-control study did relate hormone levels to esophageal adenocarcinoma [28]. Pre-operative serum testosterone levels were significantly higher in 25 male esophageal adenocarcinoma patients (median=18.2 nM/L) compared with eight age-matched patients undergoing surgery for benign conditions (median=12.5 nM/L, p=0.01). Although this may offer support for our observations, the endpoint was different. Further, post-operative (≥ 3 months) levels in these esophageal adenocarcinoma cases were reduced to levels similar to controls (median=12.2 nM/L), which led the authors to conclude that the high pre-operative levels may have been partly attributable to production by the tumor.

There is other evidence that may support our observations, particularly for the effect of free testosterone and free DHT. Individuals diagnosed with prostate cancer, who often receive some form of androgen deprivation therapy which severely reduces testosterone and DHT levels, have shown reduced risks of esophageal adenocarcinoma with standardized incidence ratios (SIR) of 0.83 (p<0.05) in a US population [29] and 0.70 (p<0.05) in a UK population [30].

Studies of reproductive factors—as proxies of hormonal exposure—in relation to esophageal adenocarcinoma have been conducted, but these had limited statistical power and all but one [31] were restricted to women [10, 26, 32-35]. Thus these prior results are unlikely to be of value in interpreting our findings here of quantitated sex steroid hormones in relation to the precursor metaplasia BE in a male population, especially given that altered levels of sex steroid hormones may have distinct effects within each sex.

There are at least three non-mutually exclusive mechanisms for our observed association with testosterone, assuming causality. The first is that testosterone and DHT are inversely associated with wound healing, possibly by inhibition of re-epithelialization [36-38], and in the esophagus this could potentially expand the interval for opportunistic metaplastic re-population. The second potential mechanism is related to inflammation. Although testosterone and DHT are generally considered anti-inflammatory [14, 39], it has also been proposed that these androgens may increase inflammation via immunosuppression [39-41]. In theory, testosterone could also undergo intra-esophageal conversion to estradiol via aromatase with subsequent pro-inflammatory effects [14], although there is scant evidence that CYP19A1 is expressed in normal esophagus (GDS1321[42], GDS3838[43]). The third possible mechanism is that testosterone could influence lower esophageal sphincter (LES) tone or frequency of transient LES relaxations, thus increasing the propensity for gastroesophageal reflux [44, 45]. In this study, adjustment for reflux symptoms had negligible effect on the association between free testosterone and BE. However, comparison with an endoscopy control group hindered our ability to assess the effect of reflux symptoms on hormone-BE associations because only 10% reported never having had daily symptoms of reflux.

With regard to the receptors for these hormones, androgen receptor (AR) protein is mostly absent from normal esophageal squamous tissue [46, 47], although one study did report positive staining in seven of 23 specimens [28]. In addition, AR gene transcription has been observed in both normal squamous epithelium [28](GEO accession: GDS3838 [43]) and—to a weaker extent—in BE (GDS3472 [48], GDS1321 [42]). More importantly, perhaps, is evidence from mice of AR up-regulation in epithelial cells, fibroblasts and macrophages following wounding [37], which may support our re-epithelialization theory. To-date, assessment of AR transcription or translation has not been assessed in erosive esophagitis patients, which may be the disease-point of interest for further assessment of this idea.

There is more consistent evidence for the presence of estrogen receptor (ER) β protein in normal squamous tissue [49-52] and in BE [53-55], and weaker evidence for ER α protein [49-52]. Gene expression data support the presence of both ER β and ER α receptors in stratified squamous epithelium and in BE (GDS4350 [56], GDS1321 [42], GDS3838 [43], GDS3472 [48]).

We did observe an inverse association between E1S concentration and BE, although no single quartile was itself statistically significant which may warrant a cautious interpretation. Prior animal and *in vitro* studies of esophageal adenocarcinoma have shown that E2 [57, 58] and 2-methoxyestradiol [59] exhibit anti-carcinogenic properties, and the normal esophagus is known to express certain 17-beta-hydroxysteroid dehydrogenases—such as HSD17B-1, 4, 5, 7, 8, 10 and 11 (GDS4350[56] GDS1321[42] GDS3472[48] GDS3838[43])—which indicates that local conversion between estrone and estradiol is possible. Animal and *in vitro* model

systems have not tested the effects of E1. The inverse association between E1S and BE and the lack of associations with E1, E2, or androgen:estrogen ratios do not support the laboratory evidence of anti-carcinogenic effects in esophageal adenocarcinoma. However, estrogen metabolism is complex and we quantitated only three metabolites of this pathway.

Strengths of this analysis include: the use of mass spectrometry to accurately quantitate steroid hormones; histologic confirmation of specialized intestinal metaplasia with goblet cells for identification of a homogeneous case group; uniform assessment at a single institute; adjustment for separate groupings of current anti-reflux medications had no effect on our estimates, despite inconsistent evidence that specific formulations of these medications may affect circulating testosterone levels [60]; and the fact that controls were recruited from the same endoscopy clinics, did not have macroscopically identifiable BE, and form the base population from which BE patients were identified for this study. Limitations include: phlebotomy was conducted after development of BE, thus we may have missed the relevant time window for disease pathogenesis; there was a small age difference between our cases and controls, although we adjusted for age in all of our analyses; endoscopy controls may not be optimal; we only quantitated our exposures once at a single age and point in time; BMI was self-reported and may not be the optimal anthropometric variable; and the study only included men seeking care thus the results may not be generalizable to non-healthcare seeking males with similar ailments.

In conclusion, we provide evidence for strong positive associations of free testosterone and free DHT with BE, which may partly explain the sex disparities of this metaplastic condition as well as esophageal adenocarcinoma. Future studies are needed to replicate this analysis, expand to population controls and esophageal adenocarcinoma cases with prediagnostic phlebotomy, and include quantitation of sex steroid hormone receptors in esophageal tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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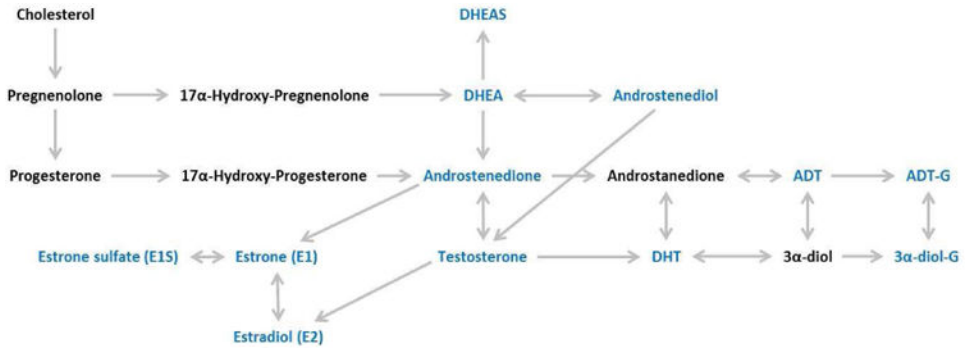

Figure. Schematic of Sex Steroid Hormone Metabolism

Sex steroid hormones that were quantitated are shown in blue font. Note that only 12 names are shown in blue yet 14 assays were conducted. This is because 3α-diol-G was quantitated as the separate metabolites of 3-androstanediol-3 glucuronide (3α-diol-3G) and 3-androstanediol-17 glucuronide (3α-diol-17G), and Sex Hormone Binding Globulin (SHBG) is not shown as it is not a part of the sex steroid biosynthesis pathway. Abbreviations: 3α-diol, 3-androstanediol glucuronide; ADT, androsterone; ADT-G, androsterone glucuronide; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulphate; DHT, dihydrotestosterone.

Table 1
Distributions of examined variables by case-control status

Variable	Controls (n=212)	Barrett's Esophagus (n=173)
Demographic Variables		
Age (years; mean (SD))	47.25 (11.88)	56.49 (11.35)
Body mass index (kg/m2; mean (SD))	28.37 (4.51)	28.41 (4.08)
Race (%)		
White	70.9	81.6
Non-white/unknown	29.1	19.4
Ever-Smoked (%)	31.5	48.0
Pack-years smoked	9.8 (2.2, 21.0)	22.0 (9.0, 47.0)
Frequency of Alcohol Use (%)		
Never/Less than monthly	15.7	16.1
Monthly	21.3	16.7
Weekly	47.7	44.4
Daily	15.2	22.8
GERD Score (out of 13)	6 (4, 8)	6 (4, 8)
GERD Score without Heartburn and Regurgitation (out of 7)	2 (2, 3)	2 (1, 3)
Ever Experienced Daily Heartburn (%)		
Never	10.3	11.0
Monthly	22.1	20.8
Weekly	29.1	28.9
Daily	38.5	39.3
Ever Experienced Daily Regurgitation (%)		
Never	10.3	13.3
Monthly	36.2	25.4
Weekly	32.4	36.4
Daily	21.1	24.9
Hormone Variables		
DHEA (nmol/L)	9.19 (5.90, 11.84)	6.33 (3.66, 9.25)
DHEAS (umol/L)	3.32 (2.25, 4.72)	2.38 (1.23, 3.93)
Androstenediol (pmol/L)	2323.6 (1715.3, 3162.9)	2004.2 (1508.8, 2877.4)
Androstenedione (nmol/L)	2.83 (2.31, 3.87)	2.69 (2.2, 3.4)
Testosterone (nmol/L)	14.1 (10.7, 18.3)	14.2 (11.2, 18.3)
DHT (pmol/L)	1107.0 (767.4, 1559.0)	1130.3 (768.7, 1442.6)
3adiol3g (nmol/L)	3.33 (2.41, 4.95)	3.36 (2.28, 4.82)
3adiol17g (nmol/L)	8.66 (6.00, 12.36)	8.87 (5.38, 11.87)
ADT (pmol/L)	803.6 (617.1, 1031.6)	656.1 (490.3, 885.3)
ADT-G (nmol/L)	78.0 (56.4, 119.0)	69.8 (45.4, 108.2)
E1 (pmol/L)	128.0 (97.5, 161.6)	123.4 (97.0, 146.0)
E1S (nmol/L)	1.58 (1.04, 2.33)	1.39 (0.82, 2.04)
E2 (pmol/L)	90.4 (73.3, 112.7)	90.7 (73.4, 107.5)

Variable	Controls (n=212)	Barrett's Esophagus (n=173)
SHBG (nmol/L)	31.5 (19.2, 44.5)	30.8 (22.3, 43.6)
Free Testosterone (nmol/L)	0.29 (0.24, 0.38)	0.29 (0.24, 0.37)
Free DHT (pmol/L)	27.13 (21.21, 35.44)	27.41 (20.91, 37.09)
Free Estradiol (pmol/L)	2.42 (1.93, 3.08)	2.32 (1.87, 2.94)

Unless otherwise stated, statistics shown are the median and interquartile range.

Table 2

Multivariable analysis of the associations between individual sex steroid hormones, SHBG and Barrett's esophagus

Variable	Controls (n=213)	Cases (n=174)	OR	95% CI	pvalue
DHEA (nmol/L)					
<5.90	52	70			
5.90-<9.20	48	48	1.06	(0.57, 2.00)	0.85
9.20-<11.85	47	18	0.57	(0.26, 1.23)	0.15
>11.85	50	23	0.93	(0.41, 2.10)	0.86
continuous	197	159	0.74	(0.46, 1.17)	0.19
DHEAS (umol/L)					
<2.25	47	67			
2.25-<3.32	48	32	0.69	(0.33, 1.41)	0.31
3.32-<4.72	44	23	0.74	(0.34, 1.63)	0.46
>4.72	43	21	1.13	(0.48, 2.64)	0.79
continuous	182	143	1.11	(0.66, 1.86)	0.70
Androstenediol (pmol/L)					
<1715.26	51	53			
1715.26-<2323.60	48	45	1.41	(0.72, 2.78)	0.32
2323.60-<3162.90	51	30	0.98	(0.48, 2.00)	0.95
>3192.9	47	29	1.62	(0.75, 3.52)	0.22
continuous	197	157	1.21	(0.72, 2.03)	0.47
Androstenedione (nmol/L)					
<2.32	48	52			
2.32-<2.83	51	36	0.64	(0.32, 1.29)	0.21
2.83-<3.87	49	44	0.93	(0.46, 1.88)	0.83
>3.87	49	27	0.71	(0.34, 1.48)	0.36
continuous	197	159	1.01	(0.63, 1.62)	0.96
Testosterone (nmol/L)					
<10.72	48	34			
10.72-<14.14	51	44	1.49	(0.72, 3.07)	0.28
14.14-<18.31	49	42	1.96	(0.92, 4.16)	0.08

Variable	Controls (n=213)	Cases (n=174)	OR	95% CI	pvalue
>18.31	49	38	2.26	(1.03, 4.95)	**0.04**
continuous	197	158	1.39	(0.76, 2.55)	0.29
DHT (pmol/L)					
<767.38	49	38			
767.38-<1107.03	49	38	1.08	(0.52, 2.22)	0.84
1107.03-<1559.02	51	51	1.53	(0.74, 3.13)	0.25
>1559.02	48	32	1.14	(0.52, 2.47)	0.75
continuous	197	159	1.19	(0.72, 1.96)	0.49
3α-diol-3G (nmol/L)					
<2.42	50	47			
2.42-<3.31	49	27	0.89	(0.43, 1.86)	0.76
3.31-<4.96	45	43	1.42	(0.70, 2.89)	0.33
>4.96	50	38	1.36	(0.66, 2.80)	0.40
continuous	194	155	0.99	(0.76, 1.28)	0.93
3α-diol-17G (nmol/L)					
<6.00	50	44			
6.00-<8.65	47	30	1.17	(0.56, 2.44)	0.67
8.65-<12.36	48	45	1.75	(0.88, 3.49)	0.11
>12.36	49	32	1.59	(0.74, 3.41)	0.23
continuous	194	151	1.02	(0.74, 1.41)	0.90
ADT (pmol/L)					
<617.13	50	77			
617.13-<803.65	52	33	0.58	(0.30, 1.15)	0.12
803.65-<1031.64	48	28	0.62	(0.31, 1.26)	0.19
>1031.64	47	21	0.79	(0.35, 1.78)	0.57
continuous	197	159	1.05	(0.63, 1.74)	0.86
ADT-G (nmol/L)					
<56.45	47	54			
56.45-<78.02	50	36	0.90	(0.45, 1.79)	0.76
78.02-<118.98	50	35	1.41	(0.68, 2.94)	0.35
>118.98	46	30	0.95	(0.46, 1.97)	0.89

Variable	Controls (n=213)	Cases (n=174)	OR	95% CI	pvalue
continuous	193	155	0.95	(0.67, 1.33)	0.76
E1 (pmol/L)					
<97.50	50	36			
97.50-<127.97	48	48	1.29	(0.64, 2.60)	0.47
127.97-<161.59	48	47	1.59	(0.79, 3.19)	0.20
>161.59	51	28	0.71	(0.33, 1.50)	0.37
continuous	197	159	0.64	(0.36, 1.14)	0.13
E1S (nmol/L)					
<0.92	45	54			
0.92-<1.49	44	33	0.54	(0.26, 1.10)	0.09
1.49-<2.17	46	29	0.70	(0.33, 1.46)	0.34
>2.17	44	29	0.48	(0.23, 1.03)	0.06
continuous	179	145	0.59	(0.38, 0.92)	**0.02**
E2 (pmol/L)					
<73.33	50	38			
73.33-<90.44	48	36	1.02	(0.49, 2.11)	0.95
90.44-<112.73	50	58	2.03	(1.02, 4.05)	**0.04**
>112.73	49	27	0.87	(0.41, 1.85)	0.73
continuous	197	159	0.99	(0.55, 1.80)	0.99
SHBG (nmol/L)					
<19.22	50	31			
19.22-<31.53	49	56	1.10	(0.54, 2.25)	0.80
31.53-<44.53	48	35	0.67	(0.30, 1.50)	0.33
>44.53	50	36	0.53	(0.24, 1.16)	0.11
continuous	197	158	0.71	(0.44, 1.14)	0.16

Adjusted for age, smoking status, alcohol consumption, heartburn, regurgitation, gastroesophageal symptom score (excluding heartburn and regurgitation), BMI, and race.

Continuous sex steroid hormone values were standardized to half of the difference between the 75th and 25th centiles of the distribution.

Table 3

Multivariable analysis of associations between combinations, ratios, and free (unbound) sex steroid hormones and Barrett's esophagus

Variable	Controls (n=212)	Cases (n=173)	OR	95% CI	p value
Parent Estrogens (pmol/L)					
<175.89	49	35			
175.89-<220.52	51	52	1.16	(0.58, 2.31)	0.67
220.52-<280.81	47	49	1.74	(0.85, 3.55)	0.13
>280.81	50	23	0.62	(0.29, 1.36)	0.23
continuous	197	159	0.73	(0.39, 1.35)	0.31
Testosterone: Parent Estrogens Ratio (pmol/L)					
<46.71	49	28			
46.71-<69.53	51	61	2.27	(1.11, 4.64)	**0.02**
69.53-<89.55	50	43	1.79	(0.83, 3.87)	0.14
>89.55	47	26	2.02	(0.86, 4.77)	0.11
continuous	197	158	1.56	(0.86, 2.83)	0.14
Androstenedione: E1 Ratio (pmol/L)					
<17.81	51	51			
17.81-<22.84	48	41	0.81	(0.41, 1.60)	0.55
22.84-<30.43	51	33	0.77	(0.38, 1.55)	0.46
>30.43	47	34	1.32	(0.62, 2.82)	0.47
continuous	197	159	1.13	(0.65, 1.94)	0.67
Testosterone: E2 Ratio (pmol/L)					
<119.31	50	30			
119.31-<160.81	49	59	1.53	(0.76, 3.09)	0.23
160.81-<205.73	50	36	1.22	(0.56, 2.64)	0.61
>205.73	48	33	1.59	(0.71, 3.54)	0.26
continuous	197	158	1.27	(0.67, 2.43)	0.47
Free Testosterone (nmol/L)					
<0.24	48	39			
0.24-<0.29	50	35	1.50	(0.73, 3.11)	0.27
0.29-<0.38	51	48	2.77	(1.30, 5.87)	**0.01**

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Variable	Controls (n=212)	Cases (n=173)	OR	95% CI	p value
>0.38	48	35	5.36	(2.21, 13.03)	**0.0002**
continuous	197	157	2.67	(1.41, 5.08)	**0.003**
Free DHT (pmol/L)					
<21.21	49	39			
21.21-<27.13	49	37	1.28	(0.63, 2.60)	0.50
27.13-<35.44	49	37	1.98	(0.93, 4.23)	0.08
>35.44	50	44	4.25	(1.87, 9.66)	**0.001**
continuous	197	157	1.98	(1.16, 3.38)	**0.01**
Free Estradiol (pmol/L)					
<1.96	50	40			
1.96-<2.42	49	46	1.35	(0.68, 2.68)	0.40
2.42-<3.09	50	45	1.38	(0.69, 2.74)	0.36
>3.09	48	27	1.25	(0.58, 2.70)	0.56
continuous	197	158	1.28	(0.68, 2.41)	0.44

Adjusted for age, smoking status, alcohol consumption, heartburn, regurgitation, gastroesophageal symptom score (excluding heartburn and regurgitation), BMI, and race. Continuous sex steroid hormone values were standardized to half of the difference between the 75th and 25th centiles of the distribution.