Title: Circulating Sex Hormone Levels and Risk of Esophageal Adenocarcinoma in a Prospective Study in Men

Running title: Sex hormones and esophageal adenocarcinoma

Authors: Shao-Hua Xie, BMed, PhD[1*], Eivind Ness-Jensen, MD, PhD[1, 2, 3*], Sirus Rabbani[1], Hilde Langseth, MSc, PhD[4], Randi E. Gislefoss, MSc, PhD[4], Fredrik Mattsson, BSc[1], Jesper Lagergren, MD, PhD[1, 5]

Affiliations: [1] Upper Gastrointestinal Surgery, Department of Molecular medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; [2] HUNT Research Centre, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Levanger, Norway; [3] Medical Department, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway; [4] Cancer Registry of Norway, Institute of Population-based Cancer Research, Department of Research, Oslo, Norway; [5] School of Cancer and Pharmaceutical Sciences, King's College London, the United Kingdom

[*] Authors contributed equally to this work.

Corresponding author: Shao-Hua Xie, BMed, PhD, Upper Gastrointestinal Surgery, Department of Molecular medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, NS 67, 2nd Floor, 17 176 Stockholm, Sweden (email: shaohua.xie@ki.se).

Abstract

Objectives: Sex hormones have been hypothesized to explain the strong male predominance in esophageal adenocarcinoma, but evidence is needed. This study examined how circulating sex hormone levels influence future risk of esophageal adenocarcinoma.

Methods: This case-control study was nested in a prospective Norwegian cohort (Janus Serum Bank Cohort), including 244 male esophageal adenocarcinoma patients and 244 male age-matched control participants. Associations between pre-diagnostic circulating levels of 12 sex hormones and risk of esophageal adenocarcinoma were assessed using conditional logistic regression. Additionally, a random-effect meta-analysis combined these data with a similar prospective study for five sex hormones.

Results: Decreased odds ratios (ORs) of esophageal adenocarcinoma were found comparing the highest with lowest quartiles of testosterone (OR=0.44, 95% confidence interval [CI] 0.22-0.88), testosterone:estradiol ratio (OR=0.37, 95% CI 0.19-0.72), and luteinizing hormone (OR=0.50, 95% CI 0.30-0.98), after adjustment for tobacco smoking and physical activity. These associations were attenuated after further adjustment for body mass index (OR=0.56, 95% CI 0.27-1.13 for testosterone; OR=0.46, 95% CI 0.23-0.91 for testosterone:estradiol ratio; OR=0.55, 95% CI 0.29-1.08 for luteinizing hormone). No associations were observed for sex hormone-binding globulin, dehydroepiandrosterone sulfate, follicle-stimulating hormone, prolactin, 17-OH-progesterone, progesterone, androstenedione, or free testosterone index. The meta-analysis showed an inverse association between testosterone levels and risk of esophageal adenocarcinoma (pooled OR for the highest versus lowest quartile=0.60, 95% CI 0.38-0.97), while no associations were identified for androstenedione, sex hormone-binding globulin, estradiol, or testosterone:estradiol ratio.

Conclusions: Higher circulating testosterone levels may decrease the risk of esophageal adenocarcinoma in men.

Study highlights

1. WHAT IS CURRENT KNOWLEDGE?

- Esophageal adenocarcinoma is characterized by an extreme and unexplained male predominance in incidence.
- Sex hormones may explain the strong male predominance in esophageal adenocarcinoma, but evidence is needed.
- How endogenous sex hormone levels influence esophageal adenocarcinoma risk warrants investigation in prospective studies.

2. WHAT IS NEW HERE?

- This was a case-control study nested in a prospective Norwegian cohort (Janus Serum Bank Cohort).
- We assessed associations between pre-diagnostic sex hormone levels and esophageal adenocarcinoma risk in men.
- Decreased esophageal adenocarcinoma risk was associated with higher testosterone, luteinizing hormone, and testosterone:estradiol ratio levels.
- These associations were attenuated after adjustment for body mass index.
- A meta-analysis confirmed a decreased risk of esophageal/gastric cardia adenocarcinoma associated with higher testosterone levels.

INTRODUCTION

The incidence of esophageal adenocarcinoma has been increasing in Western societies during the last four decades, particularly among white men [1]. The strongest risk factors for this tumor are gastroesophageal reflux disease and obesity, while infection with *Helicobacter pylori* decreases the risk [1, 2]. Esophageal adenocarcinoma is characterized by a striking male predominance, with male-to-female incidence ratios of 8-to-1 in the United States and on average 6-to-1 in Europe [3, 4]. This pattern is not explained by the main risk factors because there are no major sex differences in distribution or strengths of associations of these exposures [1, 4].

The hypothesis that sex hormonal factors explain the male predominance in esophageal adenocarcinoma is supported by a 16-year delayed onset of this cancer in women compared with men [5], and previously reported influence of sex hormonal-associated exposures, e.g. breastfeeding and reproductive factors, on the risk of esophageal adenocarcinoma [6]. However, only a few studies have examined the direct associations between circulating sex hormone levels and esophageal adenocarcinoma or its precursor lesion Barrett's esophagus [7-10]. But most of the available studies used a cross-sectional design, i.e. the blood samples used to measure sex hormone levels were collected after the disease onset, and thus, the temporal relation could not be determined [7-9]. A recent prospective study found a decreased risk of esophageal or gastric cardia adenocarcinoma associated with higher circulating levels of dehydroepiandrosterone in men [10]. The role of endogenous sex hormone levels in the etiology of esophageal adenocarcinoma requires further evaluation in prospective studies.

Based on data from a large and prospective cohort and biobank in Norway, the Janus Serum Bank Cohort, we assessed associations between circulating levels of sex hormones, which were measured in samples collected on average more than 20 years before disease onset, and the risk

of esophageal adenocarcinoma in men. Additionally, a meta-analysis combined the results from the present and previous studies.

METHODS

Study Design and Participants

This was a case-control study nested in the population-based prospective Janus Serum Bank Cohort [11]. For the purpose of the present study, serum samples and questionnaire data were retrieved from participants in five health surveys in different counties of Norway in the 1970s to 1990s, with 293 000 participants [12]. To assess the validity of the archived samples, repeated stability experiments have confirmed that relevant serum components are stable after long-term storage [13-16]. Among several variables, the questionnaire assessed tobacco smoking habits, body weight and height, and physical activity [12]. Participants were asked to report whether they were current, former, or never smokers of any tobacco product, and regular smokers were defined as those who had smoked at least one cigarette, one cigar, or one pipe per day. Body mass index (BMI) was calculated as the body weight divided by square of height (kg/m^2). Self-reported physical activity was categorized into four levels; inactive, low, medium, and high activity, according to a validated question regarding various types of exercise and physical exertion during leisure time [17]. All participants of the Janus cohort were followed up from the date when they donated the first serum sample until the date of cancer diagnosis, emigration or death, whichever occurred first. The follow-up for cancer diagnosis was enabled through linkage to the Cancer Registry and for death or emigration to the Norwegian Cause of Death Registry, using the 11-digit unique personal identification numbers assigned to all Norwegian residents. The completeness of both these registries is close to 100% [18, 19].

Because of the low incidence of esophageal adenocarcinoma in women, there were too few female cases for analysis. Therefore, this study included male participants only. By linking the Janus Serum Bank Cohort to the Cancer Registry of Norway, we identified 244 male incident

cases of esophageal adenocarcinoma until December 31, 2016. The diagnosis of esophageal cancer was determined by the diagnosis code C15 in the International Classification of Diseases, 10th version (ICD-10), and adenocarcinoma was defined based on the histological codes 8140-8141, 8143-8145, 8190-8231, 8260-8263, 8310, 8401, 8480-8490, 8550-8551, 8570-8574, or 8576, in the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) [20]. One male control participant within the Janus Serum Bank Cohort was randomly selected for each esophageal adenocarcinoma patient, using an incidence density sampling strategy from eligible participants without any cancer, except for non-melanoma skin cancer, at the time when the case was diagnosed. Each of the control participants was matched to an esophageal adenocarcinoma patient for age (± 1 year), year of blood sample drawn (± 3 months), and health survey from where the participants were recruited.

This study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (reference number 2016/1114) and was based on a broad consent from participants in the Janus cohort.

Measurements of Sex Hormone Levels

A total of 12 sex hormone measures were evaluated. The sex hormone-binding globulin and the following nine steroid sex hormones in serum samples were assessed: dehydroepiandrosterone sulfate, follicle-stimulating hormone, luteinizing hormone, prolactin, testosterone, 17-OH-progesterone, progesterone, estradiol, and androstenedione. These sex hormones cover key points in the biosynthesis of sex hormones [7, 10], are at measurable levels in serum in men and have available analytic methods in the laboratory. The free testosterone index (testosterone × 10 /

sex hormone-binding globulin) and testosterone:estradiol ratio were calculated for each participant [21]. The sex hormone levels were measured in the Hormone Laboratory, Oslo University Hospital, where the laboratory personnel were blinded to the case/control status of the study participants. Testosterone, 17-OH-progesterone, and androstenedione were analyzed by liquid chromatography - mass spectrometry, and the others were analyzed by immunoassays, all according to standard laboratory protocols.

Statistical Analysis

The descriptive statistics were computed, including means, standard deviations, quartiles, as well as minimum and maximum values, for each sex hormone comparing esophageal adenocarcinoma patients and control participants. Values of sex hormone levels below the limit of detection were imputed by the commonly used method of dividing the limit of detection by the square root of two [22]. Pairwise Spearman's correlation analysis was conducted to assess potential correlations between sex hormones. Conditional logistic regression was used to estimate odds ratio (OR) and 95% confidence interval (CI) for the association between each sex hormone and the risk of esophageal adenocarcinoma, with adjustment for covariates. The sex hormone measures were treated as categorical variables based on the cut-off values of quartiles in control participants for most hormones. Exceptions were progesterone, estradiol, and androstenedione, where over one quarter of the values were below the limit of detection; thus, levels of these sex hormones were categorized into three groups, i.e. one group of values below the limit of detection and the other two groups of approximately equal sizes for the remaining values. Because obesity might be involved in the possible causal pathway linking sex hormone levels to esophageal adenocarcinoma, two separate models were derived for each sex hormone. The main model

adjusted for tobacco smoking (never, former, or current smokers) and physical activity (high or medium, low, or inactive). A second model further adjusted for BMI (≤24.8 or >24.8, dichotomized by median value in control participants) in order to assess if BMI was involved in the mechanism behind potential associations. The statistical analyses were performed according to a pre-defined protocol, using the statistical software package SAS 9.4 (SAS Institute, Cary, NC). All statistical tests were two-sided.

Meta-analysis

A meta-analysis was performed combining the results of the present and previous prospective investigations of circulating sex hormone levels on the risk of esophageal adenocarcinoma. A systematic search of the literature in PubMed through July 19, 2019 was undertaken, supplemented by manual searches of reference lists. The search strategies in PubMed are provided in the Supplementary Materials. Only epidemiological studies meeting the following criteria were considered eligible: (1) original cohort or nested case-control study; (2) the studied outcome being the incidence of esophageal adenocarcinoma rather than mortality; (3) the exposure being circulating sex hormone levels measured before the onset of outcome; and (4) the minimum information necessary to measure the associations between sex hormone levels and the risk of esophageal adenocarcinoma and uncertainty (CI, standard error, variance, chi square and degree of freedom, or *P* value) being provided.

The methodological quality of the included studies was quantitatively assessed according to the Newcastle-Ottawa Scale, which contains eight items categorized into three domains, including

selection of participants, comparability of groups, and assessment of exposure (case-control studies) or outcome (cohort studies) [23].

The random-effect model was used to obtain pooled and adjusted ORs with 95% CIs comparing the highest with the lowest quartiles of these sex hormones. The software Comprehensive Meta-Analysis version 3 was used for the meta-analysis.

RESULTS

Participants

The mean age of blood donation was 42.2 (standard deviation [SD] ±7.2) years in esophageal adenocarcinoma patients (n=244) and 42.1 (SD ± 4.1) years in control participants (n=244). Among the esophageal adenocarcinoma patients, the mean age at diagnosis was 66.5 (SD ± 8.5) years and the mean duration from blood donation to cancer diagnosis was 24.4 (SD ± 8.5) years. Baseline characteristics of the participants are shown in Table 1. Compared with control participants, the esophageal adenocarcinoma patients had higher BMI, were more often ever smokers, and had lower levels of physical activity.

Sex Hormones and Esophageal Adenocarcinoma Risk

The distribution of circulating levels of each sex hormone in esophageal adenocarcinoma patients and in control participants is presented in Supplementary Table 1. Pairwise Spearman's correlation analysis provided the strongest coefficients for the pairings of testosterone and sex hormone-binding globulin (0.62), testosterone and testosterone:estradiol ratio (0.61), testosterone and 17-OH-progestorone (0.53), and follicle-stimulating hormone and luteinizing hormone (0.48) (Supplementary Table 2).

Higher levels of luteinizing hormone and testosterone and a higher testosterone:estradiol ratio were associated with a decreased risk of esophageal adenocarcinoma in a seemingly exposure-response manner (Table 2). The ORs comparing the highest quartile with the lowest quartile were 0.50 (95% CI 0.30 to 0.98) for luteinizing hormone, 0.44 (95% CI 0.22 to 0.88) for testosterone, and 0.37 (95% CI 0.19 to 0.72) for testosterone:estradiol ratio, after adjustment for

smoking and physical activity. These associations were attenuated after further adjustment for BMI (OR for the highest versus lowest quartile = 0.55, 95% CI 0.29 to 1.08 for luteinizing hormone; OR = 0.56, 95% CI 0.27 to 1.13 for testosterone; OR = 0.46, 95% CI 0.23 to 0.91 for testosterone:estradiol ratio). No clear associations were observed for sex hormone-binding globulin, dehydroepiandrosterone sulfate, follicle-stimulating hormone, prolactin, 17-OH-progesterone, progesterone, androstenedione, or free testosterone index (Table 2).

Meta-analysis

The literature search identified 260 articles, among which only one other nested case-control study was eligible for the meta-analysis [10]. This previous study was based on three prospective cohort studies and included 259 male patients with adenocarcinoma of the esophagus or gastric cardia and an equal number of male control participants. Both the previous and present studies had a quality score of 8 according to the Newcastle-Ottawa Scale (Supplementary Table 3). Meta-analyses were performed for the following five sex hormone measures which were included in both the present and previous studies: androstenedione, sex hormone-binding globulin, testosterone, estradiol, and testosterone:estradiol ratio. In both studies, testosterone and androstenedione were measured by mass spectrometry and sex hormone-binding globulin were measured by immunoassay. Estradiol was measured by mass spectrometry in the previous study, while immunoassay was used in the present study.

The forest plots of the ORs of esophageal or gastric cardia adenocarcinoma comparing the highest versus the lowest quartiles of sex hormone levels from the meta-analysis are shown in Figure 1. Higher levels of testosterone were associated with a decreased risk of esophageal or

gastric cardia adenocarcinoma (pooled OR = 0.60, 95% CI 0.38 to 0.97). No associations were found for androstenedione, sex hormone-binding globulin, estradiol, or testosterone:estradiol ratio (Figure 1).

DISCUSSION

This study indicates a decreased risk of esophageal adenocarcinoma associated with higher levels of luteinizing hormone and testosterone and a higher testosterone:estradiol ratio in men. No clear associations were found for sex hormone-binding globulin, dehydroepiandrosterone sulfate, follicle-stimulating hormone, prolactin, 17-OH-progesterone, progesterone, androstenedione, or free testosterone index. The meta-analysis combining the results of the present study with a previous similarly designed study confirmed a decreased risk of esophageal or gastric cardia adenocarcinoma associated with higher testosterone levels, but no associations were found for androstenedione, sex hormone-binding globulin, estradiol, or testosterone:estradiol ratio.

Strengths of this study include the population-based and prospective design, and the use of serum samples collected on average over 20 years before the diagnosis of esophageal adenocarcinoma. However, the measurement of pre-diagnostic sex hormone levels was based on a single sample only. Thus, we were not able to assess any longitudinal changes of sex hormone levels or their potential influence on the estimated associations between sex hormonal levels and risk of esophageal adenocarcinoma. The mass spectrometry method with high accuracy was used for quantitation of three sex hormones. However, the remaining sex hormones were analyzed by immunoassays with relatively lower sensitivity and specificity, which might have introduced non-differential misclassification and diluted associations. Another limitation is the lack of information on gastroesophageal reflux disease and Barrett's esophagus among the study participants. But most patients with esophageal adenocarcinoma, if not all, do have reflux and Barrett's esophagus, which makes it difficult to adjust for anyway. Moreover, reflux and Barrett's esophagus possibly part of the causal pathway from sex hormone levels to esophageal adenocarcinoma [24, 25], and therefore, adjustment for reflux or Barrett's esophagus might not

be appropriate. The statistical power was not sufficient to verify weak associations in men, but we did expect stronger associations for the hormone levels to explain the strong male predominance in esophageal adenocarcinoma.

The striking male predominance in esophageal adenocarcinoma has inspired the hypothesis of the involvement of sex hormones in the etiology, i.e. high estrogen levels may protect against this cancer and high androgen levels may increase the risk. Previous studies in women have examined the associations of exogenous hormone exposures, including hormone replacement therapy and use of oral contraceptives, or reproductive factors, including menarche, menopause, and childbearing, with the risk of esophageal adenocarcinoma, but these have mainly yielded conflicting results [1, 4]. Notably, however, a pooled analysis of three case-control studies found a strongly decreased risk of esophageal adenocarcinoma associated with increasing duration of breastfeeding, which may be related to altered sex hormone levels during pregnancy and breastfeeding [6]. Only a few studies have investigated the direct associations between circulating sex hormone levels and the risk of esophageal adenocarcinoma or Barrett's esophagus, and these have provided inconsistent findings [7-10]. Among these previous studies, three used blood samples collected from patients at diagnosis, for which potential reverse causation, i.e. sex hormone levels being influenced by the tumor, is a threat to the validity of the findings [7-9]. Only one previous study has examined the associations between pre-diagnostic levels of circulating sex hormones and the risk of esophageal or gastric cardia adenocarcinoma [10]. That study was also limited to men only and showed a decreased risk of esophageal or gastric cardia adenocarcinoma associated with higher levels of circulating dehydroepiandrosterone, estradiol, and free estradiol. The previously observed decreased risk associated with higher levels of estradiol was not supported by the results of the present study, and we did not measure

dehydroepiandrosterone or free estradiol due to the unavailability of analytic methods in the laboratory. Instead, we included several other sex hormonal measures which have not been analyzed previously.

An inverse association between testosterone levels and the risk of esophageal adenocarcinoma was indicated by both the present study and the meta-analysis that combined data from the two prospective studies. This was an unexpected finding, because the hypothesis was that higher levels of testosterone would instead increase the risk. This study also, for the first time, revealed a decreased risk of esophageal adenocarcinoma associated with higher levels of circulating luteinizing hormone. The associations between these sex hormones and the risk of esophageal adenocarcinoma need to be confirmed in more prospective studies. In addition, the associations between endogenous sex hormone levels and the risk of esophageal adenocarcinoma in women remain unknown. These associations in women need to be investigated in longitudinal studies with repeated measurements because of the varying hormone levels with the menstrual cycle in women. Such studies also need to be collaborative endeavors because of the low incidence of this tumor in women.

Sex hormones exert their biologic effects through the ligation to their receptors. The expression of androgen receptors has been found in tumor tissue of esophageal adenocarcinoma [26, 27]. Interestingly, according to the Bgee dataBase for Gene Expression Evolution, a database to retrieve and compare gene expression patterns in multiple species, the expression level of luteinizing hormone receptor is the highest in the lower esophagus, where esophageal adenocarcinoma arises, among all anatomic entities with available expression data in human [28]. However, the underlying biological mechanisms for the possible role of these hormones in the etiology of esophageal adenocarcinoma are largely unknown. Obesity may be involved in the

causal pathway because certain sex hormones, e.g. estrogen, may influence the regulation of body weight [29]. An additional hypothesis is the influence of sex hormones on inflammation, e.g. the anti- or pro-inflammation effects of by estrogen depending on the biological microenvironment, which may subsequently lead to altered cancer risk [30].

In summary, this case-control study nested in a prospective cohort and biobank indicated a decreased risk of esophageal adenocarcinoma associated with higher levels of circulating luteinizing hormone and testosterone and a higher testosterone:estradiol ratio in men. These associations were attenuated after adjustment for BMI. A meta-analysis combining data from the present study with the only other prospective study on this topic confirmed an inverse association between testosterone levels and the risk of esophageal adenocarcinoma in men. Whether sex hormones can explain the extreme sex difference in esophageal adenocarcinoma requires further large prospective investigations which include women.

REFERENCES

1. Coleman HG, Xie SH, Lagergren J. The Epidemiology of Esophageal Adenocarcinoma. Gastroenterology 2018;154:390-405.

2. Xie SH, Lagergren J. Risk factors for oesophageal cancer. Best Pract Res Clin Gastroenterol 2018;36-37:3-8.

3. Xie SH, Lagergren J. A global assessment of the male predominance in esophageal adenocarcinoma. Oncotarget 2016;7:38876-38883.

4. Xie SH, Lagergren J. The Male Predominance in Esophageal Adenocarcinoma. Clin Gastroenterol Hepatol 2016;14:338-347 e1.

5. Derakhshan MH, Liptrot S, Paul J, et al. Oesophageal and gastric intestinal-type adenocarcinomas show the same male predominance due to a 17 year delayed development in females. Gut 2009;58:16-23.

6. Cronin-Fenton DP, Murray LJ, Whiteman DC, et al. Reproductive and sex hormonal factors and oesophageal and gastric junction adenocarcinoma: a pooled analysis. Eur J Cancer 2010;46:2067-76.

7. Cook MB, Wood SN, Cash BD, et al. Association between circulating levels of sex steroid hormones and Barrett's esophagus in men: a case-control analysis. Clin Gastroenterol Hepatol 2015;13:673-82.

8. Cook MB, Wood S, Hyland PL, et al. Sex steroid hormones in relation to Barrett's esophagus: an analysis of the FINBAR Study. Andrology 2017;5:240-247.

9. Petrick JL, Falk RT, Hyland PL, et al. Association between circulating levels of sex steroid hormones and esophageal adenocarcinoma in the FINBAR Study. Plos One 2018;13:e0190325.

10. Petrick JL, Hyland PL, Caron P, et al. Associations Between Prediagnostic Concentrations of Circulating Sex Steroid Hormones and Esophageal/Gastric Cardia Adenocarcinoma Among Men. J Natl Cancer Inst 2019;111:34-41.

11. Langseth H, Gislefoss RE, Martinsen JI, et al. Cohort Profile: The Janus Serum Bank Cohort in Norway. Int J Epidemiol 2017;46:403-404g.

12. Hjerkind KV, Gislefoss RE, Tretli S, et al. Cohort Profile Update: The Janus Serum Bank Cohort in Norway. Int J Epidemiol 2017;46:1101-1102f.

13. Gislefoss RE, Grimsrud TK, Morkrid L. Long-term stability of serum components in the Janus Serum Bank. Scand J Clin Lab Invest 2008;68:402-9.

14. Gislefoss RE, Grimsrud TK, Morkrid L. Stability of selected serum proteins after long-term storage in the Janus Serum Bank. Clin Chem Lab Med 2009;47:596-603.

15. Gislefoss RE, Grimsrud TK, Hoie K, et al. Stability of testosterone measured in male archival serum samples by two different methods. Scand J Clin Lab Invest 2012;72:555-62.

16. Gislefoss RE, Grimsrud TK, Morkrid L. Stability of selected serum hormones and lipids after long-term storage in the Janus Serum Bank. Clin Biochem 2015;48:364-9.

17. Aires N, Selmer R, Thelle D. The validity of self-reported leisure time physical activity, and its relationship to serum cholesterol, blood pressure and body mass index. A population based study of 332,182 men and women aged 40-42 years. Eur J Epidemiol 2003;18:479-85.

18. Larsen IK, Smastuen M, Johannesen TB, et al. Data quality at the Cancer Registry of Norway: an overview of comparability, completeness, validity and timeliness. Eur J Cancer 2009;45:1218-31.

19. Pedersen AG, Ellingsen CL. Data quality in the Causes of Death Registry. Tidsskr Nor Laegeforen 2015;135:768-70.

20. Forman D, Bray F, Brewster DH, et al. Cancer Incidence in Five Continents, Vol. X, IARC Scientific Publication No. 164. Lyon: International Agency for Research on Cancer; 2014.

21. Carter GD, Holland SM, Alaghband-Zadeh J, et al. Investigation of hirsutism: testosterone is not enough. Ann Clin Biochem 1983;20 (Pt 5):262-3.

22. Croghan CW, Egeghy PP. Methods of Dealing with Values Below the Limit of Detection using SAS In: Southeastern SAS User Group. St. Petersburg, FL; 2003.

23. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed July 19, 2019.

24. Jacobson BC, Moy B, Colditz GA, et al. Postmenopausal hormone use and symptoms of gastroesophageal reflux. Arch Intern Med 2008;168:1798-804.

25. Nordenstedt H, Zheng Z, Cameron AJ, et al. Postmenopausal hormone therapy as a risk factor for gastroesophageal reflux symptoms among female twins. Gastroenterology 2008;134:921-8.

26. Tihan T, Harmon JW, Wan X, et al. Evidence of androgen receptor expression in squamous and adenocarcinoma of the esophagus. Anticancer Res 2001;21:3107-14.

27. Awan AK, Iftikhar SY, Morris TM, et al. Androgen receptors may act in a paracrine manner to regulate oesophageal adenocarcinoma growth. Eur J Surg Oncol 2007;33:561-8.

28. Bastian F, Parmentier G, Roux J, et al. Bgee: Integrating and Comparing Heterogeneous Transcriptome Data Among Species; 2008.

29. Brown LM, Gent L, Davis K, et al. Metabolic impact of sex hormones on obesity. Brain Res 2010;1350:77-85.

30. Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007;28:521-74.

ACKNOWLEDGMENTS

The study has used data from the Cancer Registry of Norway and the Norwegian Institute of Public Health. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway or the Norwegian Institute of Public Health is intended nor should be inferred.

CONFLICT OF INTEREST

Guarantor of the article: Shao-Hua Xie, BMed, PhD

Specific author contributions: SHX and JL jointly conceived the study. ENJ was responsible for the data collection and laboratory analyses. HL and REG contributed to the designing of study and the data collection. FM and SR performed data analysis. SHX prepared the first draft of the paper. ENJ, SR, HL, REG and JL contributed to the interpretation of the results and critically reviewed the manuscript. All authors had full access to all of the data (including statistical reports and tables) in the study and SHX can take responsibility for the integrity of the data and the accuracy of the data analysis.

Financial support: This work was supported by the Cancer Research UK (grant number C51246/A26163) to JL. The funding body had no role in the study design, the collection, analysis, and interpretation of data, or the writing of the article and the decision to submit it for publication.

Potential competing interests: None.

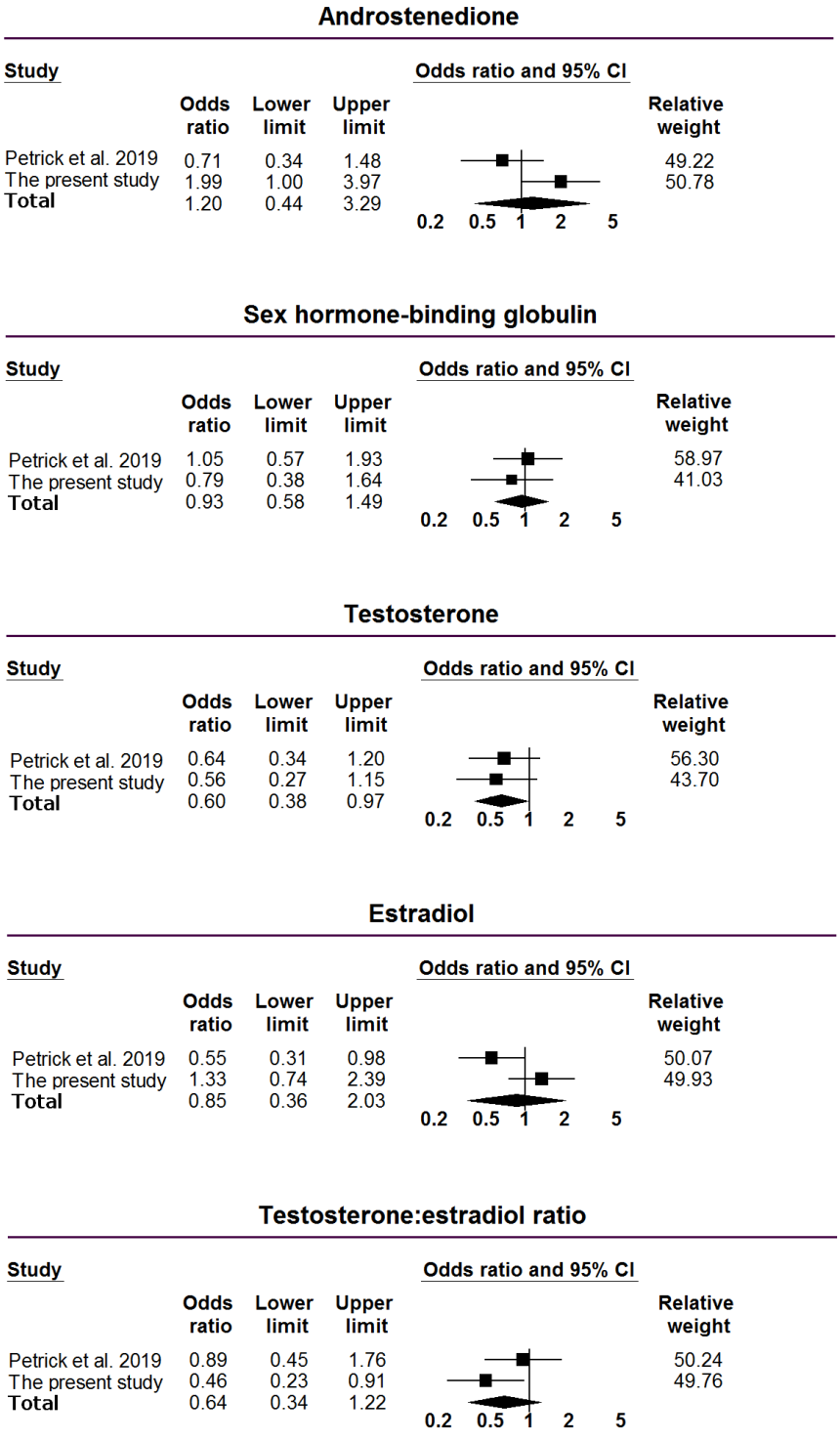

Figure 1 Forest plot of odds ratios and 95% confidence intervals (CIs) of esophageal or gastric adenocarcinoma comparing the highest versus the lowest quartiles of sex hormone levels from the meta-analysis of existing prospective studies

Table 1. Baseline characteristics of participants by case-control status, number (%)

Variable	Controls (N=244)	Cases (N=244)
Age at blood donation, years		
<40	56 (23.0)	54 (22.1)
40-49	168 (68.9)	170 (69.7)
50-59	9 (3.7)	9 (3.7)
≥60	11 (4.5)	11 (4.5)
Body mass index		
<25	123 (50.4)	84 (34.4)
25-29.9	94 (38.5)	113 (46.3)
≥30	15 (6.1)	28 (11.5)
Missing	12 (4.9)	19 (7.8)
Tobacco smoking at baseline		
Current smokers	80 (32.8)	134 (54.9)
Former smokers	68 (27.9)	50 (20.5)
Never smokers	75 (30.7)	39 (16)
Missing	21 (8.6)	21 (8.6)
Physical activity level		
Inactive	35 (14.3)	47 (19.3)
Low	117 (48.0)	120 (49.2)
Medium	73 (29.9)	54 (22.1)
High	6 (2.5)	3 (1.2)
Missing	13 (5.3)	20 (8.2)

Table 2. Association between circulating sex hormone levels and risk of esophageal adenocarcinoma

Sex hormone	Number of controls	Number of cases	Odds Ratio (95% CI) *	Odds Ratio (95% CI) †
Sex hormone-binding globulin, nmol/L				
<28.0	53	49	Reference	Reference
28.0 to <41.0	61	87	1.23 (0.67 to 2.25)	1.29 (0.69 to 2.41)
41.0 to <53.0	65	50	0.67 (0.35 to 1.29)	0.77 (0.39 to 1.52)
≥53.0	61	54	0.66 (0.33 to 1.32)	0.79 (0.38 to 1.64)
P for trend			0.032	0.143
Dehydroepiandrosterone sulfate, µmol/L				
<4.50	56	56	Reference	Reference
4.50 to <6.20	63	47	0.52 (0.27 to 1.03)	0.57 (0.28 to 1.14)
6.20 to <8.25	61	65	0.82 (0.44 to 1.52)	0.79 (0.42 to 1.49)
≥8.30	60	72	0.85 (0.45 to 1.63)	0.89 (0.46 to 1.71)
P for trend			0.782	0.794
Follicle stimulating hormone, IU/L				
<2.7	58	56	Reference	Reference
2.7 to <3.8	59	55	1.31 (0.71 to 2.39)	1.22 (0.65 to 2.27)
3.8 to <5.2	62	67	1.33 (0.74 to 2.40)	1.25 (0.68 to 2.29)
≥5.2	61	62	1.31 (0.61 to 2.10)	1.00 (0.53 to 1.90)
P for trend			0.909	0.831
Luteinizing hormone, IU/L				
<3.25	60	59	Reference	Reference
3.25 to <4.70	59	60	0.93 (0.51 to 1.68)	1.13 (0.60 to 2.12)
4.70 to <6.3	60	71	0.82 (0.45 to 1.51)	1.00 (0.53 to 1.89)
≥6.3	61	50	0.50 (0.26 to 0.98)	0.55 (0.29 to 1.08)
P for trend			0.035	0.052
Prolactin, mIU/L				
<110.0	59	83	Reference	Reference
110.0 to <158.0	60	56	0.73 (0.41 to 1.29)	0.73 (0.41 to 1.31)
158.0 to <228.5	61	59	0.80 (0.44 to 1.45)	0.82 (0.45 to 1.52)
≥228.5	60	42	0.57 (0.30 to 1.10)	0.58 (0.30 to 1.13)
P for trend			0.126	0.148
Testosterone, nmol/L				
<12.2	55	65	Reference	Reference
12.2 to <16.3	64	64	0.71 (0.38 to 1.30)	0.83 (0.44 to 1.55)
16.3 to <20.5	59	54	0.53 (0.27 to 1.03)	0.69 (0.35 to 1.39)
≥20.5	61	57	0.44 (0.22 to 0.88)	0.56 (0.27 to 1.13)
P for trend			0.017	0.095

17-OH-progesterone, nmol/L				
<1.3	51	50	Reference	Reference
1.3 to <1.8	61	54	0.73 (0.38 to 1.39)	0.70 (0.36 to 1.36)
1.8 to <2.6	64	71	1.01 (0.54 to 1.89)	1.14 (0.59 to 2.21)
≥2.6	63	65	0.71 (0.37 to 1.37)	0.86 (0.43 to 1.72)
P for trend			0.447	0.967
Progesterone, nmol/L				
<1.5	167	153	Reference	Reference
>1.5 to 1.7	38	45	1.21 (0.69 to 2.12)	1.27 (0.72 to 2.25)
>1.7	35	42	1.16 (0.64 to 2.10)	1.37 (0.74 to 2.55)
P for trend			0.557	0.272
Estradiol, nmol/L				
<0.07	102	70	Reference	Reference
0.07 to <0.08	63	95	2.14 (1.27 to 3.62)	2.06 (1.21 to 3.51)
≥0.09	75	75	1.35 (0.76 to 2.39)	1.33 (0.74 to 2.38)
P for trend			0.312	0.342
Androstenedione, nmol/L				
<2.7	114	82	Reference	Reference
2.7 to <4.2	64	74	1.66 (0.99 to 2.78)	1.64 (0.96 to 2.78)
≥4.2	61	84	2.00 (1.02 to 3.94)	1.99 (1.00 to 3.98)
P for trend			0.094	0.103
Testosterone:estradiol ratio				
<180.0	58	80	Reference	Reference
180.0 to <228.3	61	52	0.53 (0.29 to 0.98)	0.56 (0.30 to 1.06)
228.3 to <306.4	60	71	0.65 (0.36 to 1.17)	0.73 (0.40 to 1.34)
≥306.4	60	37	0.37 (0.19 to 0.72)	0.46 (0.23 to 0.91)
P for trend			0.007	0.047
Free testosterone index				
<3.33	59	60	Reference	Reference
3.33 to <4.07	60	54	0.94 (0.52 to 1.70)	1.02 (0.55 to 1.88)
4.07 to <5.10	60	64	1.00 (0.54 to 1.90)	1.08 (0.57 to 2.03)
≥5.10	60	62	0.89 (0.46 to 1.75)	0.90 (0.45 to 1.79)
P for trend			0.757	0.726

[*] Adjusted for tobacco smoking (never, former, or current smokers) and physical activity (high or medium, low, or inactive).

[†] Further adjusted for body mass index at baseline (≤24.8, or >24.8).

CI: confidence interval.

Search strategies in PubMed

1. estrogen OR oestrogen OR androgen OR testosterone OR (dehydroepiandrosterone sulfate) OR (dehydroepiandrosterone sulphate) OR (follicle stimulating hormone) OR (luteinizing hormone) OR (luteinising hormone) OR prolactin OR progesterone OR estradiol OR androstenedione OR FSH OR LH OR (sex hormone binding globulin) OR SHBG OR (sex hormone*) OR (steroid hormone*)

2. esophagus OR oesophagus OR esophageal OR oesophageal OR (upper digestive) OR (upper gastrointestinal) OR (upper aerogidestive)

3. cancer OR carcinoma OR adenocarcinoma OR tumor OR tumour OR malignan* OR neoplas*

4. 1 AND 2 AND 3

All terms were searched in TITLE/ABSTRACT.

Supplementary Table 1. Distributions of circulating sex hormone levels by case-control status

Hormone	Group	Mean	Standard deviation	Median	Lower quartile	Upper quartile	Minimum	Maximum	Missing
Sex hormone-binding globulin, nmol/L	Controls	42.00	16.87	41.00	28.00	53.00	7.00	103.00	4
	Cases	40.90	15.52	38.50	29.00	51.00	12.00	89.00	4
Dehydroepiandrosterone sulfate, µmol/L	Controls	6.70	3.18	6.20	4.5.00	8.25	0.80	25.50	4
	Cases	7.11	3.43	6.90	4.60	8.80	0.28	21.20	4
Follicle-stimulating hormone, IU/L	Controls	4.61	4.34	3.80	2.70	5.20	1.20	56.50	4
	Cases	4.72	3.47	3.90	2.70	5.20	0.71	32.20	4
Luteinizing hormone, IU/L	Controls	5.06	2.67	4.70	3.25	6.30	1.60	24.80	4
	Cases	4.98	2.49	4.70	3.30	6.10	1.50	25.60	4
Prolactin, mIU/L	Controls	188.66	143.50	158.00	110.00	228.50	19.00	1243.00	4
	Cases	169.35	189.56	141.00	96.00	198.00	24.00	2690.00	4
Testosterone, nmol/L	Controls	17.00	7.09	16.30	12.20	20.50	1.20	53.30	5
	Cases	16.57	6.79	15.50	11.80	20.15	1.70	47.70	4
17-OH-progesterone, nmol/L	Controls	2.08	1.05	1.80	1.30	2.60	0.14	5.90	5
	Cases	2.13	1.15	1.90	1.30	2.60	0.14	8.90	4
Progesterone, nmol/L	Controls	1.31	0.44	1.06	1.06	1.50	1.06	3.40	4
	Cases	1.36	0.47	1.06	1.06	1.60	1.06	3.60	4
Estradiol, nmol/L	Controls	0.07	0.03	0.07	0.04	0.09	0.04	0.16	4
	Cases	0.08	0.03	0.08	0.06	0.09	0.04	0.16	4
Androstenedione, nmol/L	Controls	5.05	6.02	2.80	1.91	4.40	1.91	33.00	5
	Cases	5.39	5.77	3.20	1.91	5.15	1.91	33.70	4
Testosterone:estradiol ratio	Controls	247.25	100.89	228.33	180.00	306.41	28.28	688.25	5
	Cases	228.30	95.58	218.66	165.28	273.53	24.29	669.39	4
Free testosterone index	Controls	4.37	2.29	4.07	3.33	5.10	0.52	31.35	5
	Cases	4.26	1.44	4.16	3.33	5.17	0.35	8.94	4

Supplementary Table 2. Pair-wise Spearman's correlation coefficients between circulating sex hormone levels

Hormone	SHBG	DHEAS	FSH	LH	Prolactin	Testosterone	17-OHP	Progesterone	Estradiol	AE	T:E2 ratio	FTI
SHBG	1	-0.02	0.09	0.27**	0.13**	0.62**	0.23**	0.02	0.19**	0.08	0.47**	-0.42**
DHEAS	-0.02	1	-0.05	0.01	0.06	0.08	-0.04	0.45**	0.03	0.15**	0.04	0.12**
FSH	0.09	-0.05	1	0.48**	0.08	0.07	-0.04	-0.02	0.06	0.02	0.04	-0.01
LH	0.27**	0.01	0.48**	1	0.12**	0.28**	0.19*	0.07	0.26**	0.13**	0.06	0.01
Prolactin	0.13**	0.06	0.08	0.12**	1	0.06	-0.03	-0.08	0	0.02	0.02	-0.11*
Testosterone	0.62**	0.08	0.07	0.28**	0.06	1	0.53**	0.09	0.43**	0.25**	0.61**	0.39**
17-OHP	0.23**	-0.04	-0.04	0.19**	-0.03	0.53**	1	0.14**	0.25**	0.32**	0.30**	0.38**
Progesterone	0.02	0.45**	-0.02	0.07	-0.08	0.09	0.14**	1	0.10*	0.16**	0.02	0.07
Estradiol	0.19**	0.03	0.06	0.26**	0	0.43**	0.25**	0.10*	1	0.17**	-0.39**	0.28**
AE	0.08	0.15**	0.02	0.13**	0.02	0.25**	0.32**	0.16**	0.17**	1	0.12*	0.19**
T:E2 ratio	0.47**	0.04	0.04	0.06	0.02	0.61**	0.30**	0.02	-0.39**	0.12*	1	0.14**

* P<0.05; ** P<0.01

AE: androstenedione; DHEAS: dehydroepiandrosterone sulfate; FSH: follicle stimulating hormone; FTI: free testosterone index; LH: luteinizing hormone; 17-OHP: 17-OH-progesterone; SHBG: sex hormone-binding globulin; T:E2 ratio: testosterone:estradiol ratio

Supplementary Table 3. Quality assessment scale of studies included in the meta-analysis

Study	Selection				Comparability	Exposure			Total
	Case definition	Representativeness of cases	Selection of controls	Control definition		Exposure ascertainment	Ascertainment method	Non-response rate	
Petrick et al. 2019	0	1	1	1	2	1	1	1	8
The present study	0	1	1	1	2	1	1	1	8