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# Increased levels of enzymes involved in local estradiol synthesis in chronic obstructive pulmonary disease

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ABSTRACT

*Introduction:* Steroid hormones are involved in lung development, pulmonary inflammation, and lung cancer. Estrogen signaling and exposure may play a role in pulmonary disorders, including COPD. In both genders, estrogens can be generated locally in the lungs and this contributes importantly to the tissue exposure to these steroids.
*Objective:* To characterize and assess differences in localization of estrogen receptors and enzymes involved in the local generation of estrogens in COPD.
*Methods:* Estrogen Receptor alpha (ERα/ESR1), Estrogen Receptor beta (ERβ/ESR2) and G-protein-coupled estrogen receptor 1 (GPER) were explored by real-time (RT)-PCR analysis (mRNA expression), immunohistochemistry and western blotting in controls and COPD patients.
mRNA expression of the enzymes involved in the local estrogen generation – i.e. aromatase (CYP19A1), 17beta-hydroxysteroid dehydrogenases (17β-HSDs) 1, 2, 4, 5, 7 and 12, steroid sulfatase (STS) and sulfotransferase (SULT1E1) - were analyzed by RT-PCR.
*Results:* ERα, ERβ and GPER were expressed in lung tissue, but no differences were observed between patients and controls. The main enzymes involved in local estrogen generation were also present in both normal and COPD lung tissue. In lungs of COPD patients compared with controls, we observed increased expression of the enzymes 17β-HSD type 1 and aromatase (positive association), both involved in the local synthesis of active estrogens.
*Conclusion:* All ER subtypes are present in the lung. The shift in local mRNA level of estrogen metabolic enzymes suggests that exposure to estrogens is involved in the pathogenesis of COPD.


## 1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common and debilitating chronic health condition characterized by progressive and persistent airflow limitation, associated with an enhanced chronic pulmonary inflammatory response. The major risk factor for disease development is exposure to inhaled noxious particles or gases, such as tobacco smoke (Hogg and Timens, 2009; Rabe et al., 2007; Vestbo et al., 2013). The prevalence of COPD is nearly equal among men and women, but the incidence of the disease is increasing more rapidly in women than in men. Gender disparities amongst various lung diseases are commonly reported and biological and clinical evidence suggests a contribution of the female hormones estrogens in disease development (Ben-Zaken Cohen et al., 2007; Han et al., 2007; Sathish et al., 2015; Tam et al.,; Townsend et al., 2012).

The final cellular and tissue effects exerted by estrogens depend on the presence of the ligand-activated estrogen receptors (ERs, namely ERα, ERβ and G Protein-coupled estrogen receptor, GPER) and the intracellular availability of estrogens, i.e. the ligand for the receptors (Gustafsson, 2003; Heldring et al., 2007). ERα and ERβ

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activate the classical genomic and non-genomic estrogen signaling mechanisms, i.e., they directly or indirectly (via interaction with other transcription factors) control the transcription of target-genes. The more recently characterized transmembrane GPER mediates rapid non-genomic signaling (Carmeci et al., 1997; Jala et al., 2012; Olde and Leeb-Lundberg, 2009; Prossnitz et al., 2008; Zhou et al., 2015) and was recently implicated in genomic signaling as well (Zhou et al., 2015).

Before menopause, the major sources of estrogens are the ovaries, which contribute largely to the circulating levels of these hormones. However, in postmenopausal women and in men, estrogens are generated locally in various reproductive and non-reproductive tissues using as precursors serum androgens and adrenal-cortex steroids. This extra-ovarian source of estrogens controls the final intracellular availability of the ligand for the ERs (Delvoux et al., 2014; Labrie and Labrie, 2013; Luu-The and Labrie, 2010). Such local generation of estrogens is controlled by enzymatic conversions (Labrie, 2003), among which, one of the most important is the formation of estradiol (E2), the natural and most potent estrogen, from estrone (E1), a precursor of E2 with little biological activity. The enzymes 17-beta-hydroxysteroid dehydrogenases (17β-HSDs) control the interconversion between E1 and E2. Type 1 17β-HSD catalyzes the reduction of E1 to E2, whereas type 2 17β-HSD catalyzes the oxidation of E2 to E1 (Payne and Hales, 2004). A number of additional reductive (types 5, 7 and 12) and oxidizing (type 4) 17β-HSDs have been suggested to activate and deactivate estrogens. Although a potential role of 17β-HSD types 7 and 12 to convert E1 into E2 is not fully excluded (Wang et al., 2015), recent investigations disregard that the remaining enzymes can use these steroids as substrates *in vivo* (Cornel et al., 2012; Miller and Auchus, 2011; Moeller and Adamski, 2009).

Beside the redox balance between E1 and E2 controlled by types 1 and 2 17β-HSDs, steroid sulfatase (STS) and estrogen sulfotransferase (SULT1E1) are involved in local estrogen production. E1 conjugated with a sulfate group (E1-sulfate) is the estrogenic compound with the highest concentration in the blood due to its high water solubility and long half-life. E1-sulfate is completely inactive and is not able to pass the cell membrane. STS is a membrane-associated microsomal enzyme localized on the endoplasmic reticulum (Mueller et al., 2015) and converts E1-sulfate to free intracellular E1, while SULT1E1 catalyzes the reverse reaction. A final extra-ovarian source of estrogens is represented by the aromatization of the androgens androstenedione (A) and testosterone (T) to E1 and E2, respectively, catalyzed by the enzyme CYP19A1 aromatase (Cornel et al., 2012; Miller and Auchus, 2011; Moeller and Adamski, 2009).

Recent investigations underscore the role of these local metabolic conversions in causing local estrogen overexposure. A shift in the estrogen metabolism favoring the generation of E2 has been observed in several benign and malignant gynecological disorders (Cornel et al., 2012; Delvoux et al., 2014; Delvoux et al., 2009), and also in lung diseases (Drzewiecka et al., 2015; Niikawa et al., 2008; Sin et al., 2007). In non-small cell lung cancer both aromatase (Niikawa et al., 2008) and 17β-HSD type 1 (Agrawal and Verma, 2013; Drzewiecka et al., 2015) contribute to an increased intratumoral generation of E2. Furthermore, in interstitial pneumonia, local E2 concentrations in alveolar epithelial cells are increased due to elevated levels and activity of aromatase (Taniuchi et al., 2014).

The contribution of local E2 in COPD has not been explored to date. However, women have a higher predisposition to develop the disease (Sin et al., 2007), and, although controversial, epidemiological studies have shown that hormone replacement therapy containing estrogens exacerbates COPD (Barr and Camargo, 2004).

In this study we examined whether the estrogen signaling and the intracellular pathways controlling the generation of E2 are associated with COPD. We examined the level of expression and cellular localization of ER subtypes and of the major estrogen metabolizing enzymes in COPD affected and control lung tissue. Men and women (in most cases after menopause) were analysed together because it is known that the serum level of estrogens and their main precursors show only small differences between men and postmenopausal women (http://www.glowm.com), and the generation of these steroids locally from serum substrates represents the major estrogen source.

## 2. Material and methods

### 2.1. Ethical statement

The present study was conducted in compliance with the Helsinki Declaration. Protocols to use human materials for research were approved by the competent medical ethical authorities as stated below.

### 2.2. Study population 1

Lung tissue for quantitative Real-Time PCR (RT-PCR) and western blot analyses was obtained from 50 patients diagnosed with solitary pulmonary tumors at the Ghent University Hospital, Belgium. The subjects of study population 1 (Table 1) consisted of 24 COPD patients and 26 subjects without COPD (controls). Tissue specimens were obtained from the subpleural area of the upper lobe distant from the tumor. This study population was previously described (Verhamme et al., 2014). All subjects provided written informed consent, according to protocols approved by the medical ethical committee of the Ghent University Hospital.

### 2.3. Study population 2

In a second group of patients (population 2; Table 1), lung tissue for immunohistochemistry with a cross-sectional surface of approximately 2 $cm^2$ was obtained at the University hospital Maastricht, the Netherlands. The lung resection specimens for immunohistochemistry were obtained from tumor-free lung tissue in the subpleural area from 49 COPD patients, and from 22 subjects without COPD who underwent resection for a solitary peripheral tumor. Signs of a respiratory tract infection during four weeks preceding the study and a history of respiratory diseases, other than lung cancer were considered exclusion criteria. Anonymized archival lung tissue was obtained from the Maastricht Pathology Tissue Collection (MPTC). Collection, storage and use of tissue and patient data were performed in agreement with the “Code for Proper Secondary Use of Human Tissue in the Netherlands”. The scientific board of the MPTC approved the use of materials for this study under MPTC 2009-22.

### 2.4. Clinical characteristics of study populations

The clinical characteristics of study population 1 and 2 were collected from hospital records and are summarized in Table 1. Both men and women were included: 36 men and 14 women were present in population 1 (two women were below the age of 55 and likely peri/premenopausal); 38 men and 33 women were present in population 2 (eight women were below 55, and likely peri/premenopausal). Analyses were corrected for both gender and menopausal status, as described below. The number of pack-years and the smoking status were recorded. All subjects had smoked at least 10 pack-years. Subjects who stopped smoking at least one year prior to recruitment were considered ex-smokers. Lung function was determined by spirometry. Post-bronchodilator