From the Unit of Esophageal and Gastric Research, Department of Molecular Medicine and Surgery

ESTROGEN IN THE DEVELOPMENT OF ESOPHAGEAL AND GASTRIC ADENOCARCINOMA

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Στους γονείς μου,
στην Αλεξάνδρα και
στην Ζανθή
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<td>AC</td>
<td>Adenocarcinoma</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>E₁</td>
<td>Estrone</td>
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<td>E₂</td>
<td>17β-estradiol</td>
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<td>E₃</td>
<td>Estriol</td>
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<td>ER</td>
<td>Estrogen receptor</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GP</td>
<td>General practitioner</td>
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<tr>
<td>GPRD</td>
<td>General Practice Research Database</td>
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<td>H. pylori</td>
<td>Helicobacter pylori</td>
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<td>HRT</td>
<td>Hormone replacement therapy</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
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<td>ICD-O</td>
<td>International Classification of Diseases for Oncology</td>
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<tr>
<td>MNNG</td>
<td>N-methyl-N’-nitro-N-nitrosoguanidine</td>
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<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>SIR</td>
<td>Standardized incidence ratio</td>
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<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
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<td>SERM</td>
<td>Selective estrogen receptor modulator</td>
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<td>WHI</td>
<td>Women’s Health Initiative</td>
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List of Papers

This thesis is based on the following papers, which will be referred to by their Roman numerals

I. Lindblad M, García Rodríguez LA, Chandanos E, Lagergren J
Hormone replacement therapy and risks of oesophageal and gastric adenocarcinomas

II. Chandanos E, Lindblad M, Jia C, Rubio CA, Ye W, Lagergren J
Tamoxifen exposure and risk of oesophageal and gastric adenocarcinoma: a population-based cohort study of breast cancer patients in Sweden

Endogenous estrogen exposure in relation to distribution of histological type and estrogen receptors of gastric adenocarcinoma
(submitted)

IV. Chandanos E, Lindblad M, Rubio CA, Jia C, Warner M, Gustafsson JÅ, Lagergren J
Tamoxifen exposure in relation to distribution of histological type and estrogen receptors of gastric adenocarcinoma
(submitted)
Abstract

The aim of this thesis was to address the hypothesis that estrogen protects women against the development of esophageal and gastric adenocarcinoma (AC). These two cancers show a striking but totally unexplained male predominance in their incidence. There are 6-7 men for every woman affected by esophageal AC, while the male: female ratio in gastric AC is 2-3:1.

In the first study, we investigated whether hormone replacement therapy (HRT) with estrogen protects postmenopausal women against esophageal and gastric AC. We conducted a nested case-control study, in which cases and controls came from a cohort of postmenopausal women in the United Kingdom. Prospectively recorded data from the General Practice Research Database were used. Women on HRT had a significantly decreased risk of gastric AC (odds ratio (OR) 0.48, 95% confidence interval (CI) 0.29-0.79), an association that seemed stronger for non-cardia than for cardia gastric AC (OR 0.34, 95% CI 0.14-0.78). HRT use was not linked with a decreased risk of esophageal AC (OR 1.17, 95% CI 0.41-3.32).

In the second study, we prospectively assessed the risk of esophageal and gastric AC in women who had been treated for breast cancer with the anti-estrogen drug tamoxifen. Our large population-based cohort of postmenopausal women was identified from the Swedish Cancer Register. Among 138 885 cohort members contributing to more than 1 million person-years of follow-up, we found a non-significantly increased risk of esophageal AC in those exposed to tamoxifen (standardized incidence ratio (SIR) 1.60, 95% CI 0.83-3.08). No association was observed in the unexposed. No increased risk of cardia AC was identified, irrespective of exposure to tamoxifen. The risk of non-cardia gastric AC was increased in the tamoxifen-exposed cohort (SIR 1.27, 95% CI 1.03-1.57), and it was almost doubled (SIR 1.86, 95% CI 1.10-3.14) in the period of longest latency (10-14 years) after breast cancer diagnosis. We concluded that there might be a link between tamoxifen and risk of non-cardia gastric AC.

In the third and fourth studies the potential influence of endogenous estrogen and of tamoxifen exposure on the risk of the intestinal type of gastric AC was addressed. Immunohistochemical laboratory work was conducted in order to assess the expression of estrogen receptors (ERs). In both studies we used data from the Swedish Cancer Register and we identified patients with gastric cancer diagnosed in the county of Stockholm. In the third study we categorized gastric AC cases into three groups according to their endogenous estrogen exposure: 1) women aged <50 years ("exposed women"), 2) men <50 years ("unexposed men") and 3) women >70 years ("unexposed women"). Compared to "exposed women", the intestinal type of gastric AC was over 4 and 9 times more common among "unexposed men" (OR 4.7; 95% CI 2.2-10.3) and "unexposed women" (OR 9.1; 95% CI 4.3-19.6), respectively. No differences in ER expression were found between the three groups. A loss of ERbeta and a gain of ERalpha in the tumor cells compared to non-tumor cells was observed. ERbeta cx was identified for the first time in gastric tissue.

In the fourth study we identified all women with a breast cancer diagnosis who subsequently developed gastric AC. The intestinal type of gastric AC was not more frequent among tamoxifen users (27%) than among non-users (34%) (p=0.601). There were no material differences between the two groups regarding distribution of ERs in intestinal AC specimens. Tamoxifen users seemed to have a shorter latency between breast cancer and gastric AC diagnoses.

In conclusion, this thesis supports the hypothesis that estrogen may protect women against development of gastric AC, while we did not find evidence for this in esophageal AC. The mechanism underlying this protection is not yet clear, but it may be mediated by ERs. For the first time the existence of estrogen receptor beta cx in gastric tissue is reported.
Cancer is one of the oldest recognized diseases of mankind. The first description of cancer appeared in Egyptian papyri in 1500 B.C. About 1000 years later Hippocrates observed that tumors have a characteristic growth pattern, resembling the claws of a crab. He named the disease “karkinos” from the Greek word for crab, which in English translates to cancer or carcinoma. Today cancer consists of more than 100 diseases that can affect any tissue in the body.

According to the World Health Organization, more than 7.5 million cancer deaths (approximately 13% of all deaths) occurred worldwide in the year 2005. It is predicted that the burden of cancer in humans will continue to rise, and the global estimate of cancer deaths in the year 2015 is 9 million. The five most common killing forms of cancer in men are lung, stomach, liver, colorectal and esophageal cancer, while in women they are breast, lung, stomach, colorectal and cervical cancer.

Esophageal adenocarcinoma is the most rapidly increasing form of cancer in Western populations, while in these populations the incidence of adenocarcinoma of the stomach is decreasing. There is a clear, but totally unexplained, male predominance in the incidence of adenocarcinoma of the esophagus and stomach; in some areas of the world up to 8 men for every woman are affected by esophageal adenocarcinoma, while the incidence of gastric adenocarcinoma is at least twice as common in men. These ratios cannot be explained on the basis of sex differences in the prevalence of known risk factors. Despite advancements in the diagnosis and treatment of cancer in general, the prognosis of esophageal and gastric cancer remains dismal. Thus, research focusing on the etiology of these cancer forms is of paramount importance; understanding of the causes may lead to the development of effective preventive measures, better surveillance, and improved treatment.

In this thesis, consisting of four original studies, the hypothesis that estrogen protects women against esophageal and gastric adenocarcinoma was investigated. Both epidemiological studies and laboratory work were conducted for this purpose.
Estrogen in the Development of Esophageal and Gastric Adenocarcinoma
Background

Esophageal Adenocarcinoma

Incidence and trends

Esophageal cancer is the eighth most common cancer worldwide, with an incidence of more than 460,000 cases and almost 400,000 deaths in 2002, making it the sixth most common form of cancer death globally. The most recently available data in Sweden show an incidence of 432 cases in 2005. Of these, 326 were diagnosed in men and 106 in women.

There are two main histological types of esophageal cancer: squamous cell carcinoma (SCC) and adenocarcinoma (AC), both derived from the epithelial cells that cover the inner layer (mucosa) of the esophagus. Together these types constitute more than 90% of all cases, while the rest are rare histological types: melanomas, leiomyosarcomas, lymphomas, and carcinoids. Adenocarcinoma originate from glandular epithelial tissue and mainly occurs in the lower third of the esophagus.

Up to a few decades ago only a low percentage of esophageal cancers were AC. In an American study of 1859 patients diagnosed with esophageal cancer between 1926 and 1966, only 45 (2%) had AC. In another study based on 25 years of experience from a single institution in the United States, an AC incidence of 4% was found, and a review of the literature by the authors revealed the same incidence among 6673 cases of esophageal cancer. Similar results for the period 1945-1969 have been reported from Scandinavia. During the last two decades, however, a rapid increase in the incidence of AC has been observed, particularly among males. The increase has been most notable among Caucasians and by the mid-1980s AC constituted one third of all cases of esophageal cancer in that group in the United States. The incidence rose by more than 350% in the mid-1970s and surpassed that of SCC around 1990.

In Scandinavia as well as in other parts of the world, similar trends have been reported. In Sweden, in a period of about two decades the age-standardized incidence rate of AC was more than doubled in men, while the increase in the incidence of SCC was less pronounced. The incidence of esophageal AC in Sweden in the period 1970-2005 is depicted in Figure 1. There is a clear increase in its incidence, which is much more evident in men. Moreover it is seen that there has been a clear and constant male predominance since the mid-1970s, with a dramatic increase since the 1990s.
It has been discussed whether this increase in AC is real or has just been observed coincidentally.\textsuperscript{24} For example, with the advancement of computer tomography since the mid-1970s\textsuperscript{26} and the improvements and widespread availability of endoscopy since the 1980s,\textsuperscript{27} cancer may in recent years have been detected earlier than before (lead-time bias) giving the false impression that there has been an increase in its incidence. Public awareness of the disease, shorter patient delay in seeking medical help, and tumor misclassification have also been discussed as potential misleading factors regarding the increased number of cases. These cannot, however, explain the rise which has continued beyond a temporary increase that one would expect in the event of better diagnostic modalities and greater general awareness. Moreover, the trend differs between subgroups of a population (women vs. men, Caucasians vs. blacks). In the event of inaccurate diagnosis and misclassification a change in the incidence of other esophageal tumors should have been noted. The slight decrease in the incidence of esophageal squamous cell carcinoma\textsuperscript{28} does not account for the striking increase in AC.\textsuperscript{28,29} In addition, cardia gastric AC, which is a proximal gastric cancer that is difficult to distinguish from esophageal AC as it is located in the transition area between the esophagus and the stomach,\textsuperscript{30} is also showing an increasing incidence.\textsuperscript{9-11, 20, 22, 31} Even if some degree of misclassification with regard to esophageal cancer occurs in the Swedish Cancer Register, which has an excellent completeness rate,\textsuperscript{30,32} the observed increase in the incidence of esophageal AC cannot be explained by tumor misclassification.\textsuperscript{30} Thus, the increase in esophageal AC cases must be real.

**Prognosis**

Although much effort has been put into diagnosing and treating esophageal cancer, the prognosis, compared to the five most killing forms of cancer worldwide (in men: lung, stomach, liver, colorectal and esophageal; in women: breast, lung, stomach, colorectal and cervix), is only slightly better than that of liver cancer and as bad as that of lung cancer.\textsuperscript{1} The 5–year survival rate is between 10 and 16% in the Western countries,\textsuperscript{33,34} including Sweden.\textsuperscript{35} The prognosis of AC in Sweden has improved in recent years. The five-year observed survival rate rose from 4% during 1961-1989 to 15.5% during 1990-1996.\textsuperscript{35}

**Etiology**

Great efforts have been concentrated on investigating the etiology of esophageal AC. Heredity plays no material role. Barrett’s esophagus, a condition in which the normal squamous epithelium that lines the distal esophagus has been replaced by columnar epithelium,\textsuperscript{40} has been linked
to esophageal AC. Gastroesophageal reflux is an independent and dose-dependent risk factor per se. Another strong and dose-dependent association has been found between high body mass index (BMI) and esophageal AC, a finding of paramount importance, as the prevalence of obesity is increasing worldwide. Tobacco smoking has been shown to be positively associated with esophageal AC, but this link is only of moderate strength. As mentioned above, the incidence of this cancer among white males is increasing, despite the falling incidence of lung cancer, which has a much stronger association with tobacco smoking. Thus as a risk factor smoking plays a more limited role in the development of esophageal AC. Results of studies on alcohol drinking as a risk factor for esophageal AC have been conflicting; some researchers have found no association, while another group has reported a protective effect of wine. Also contradictory are the results of investigations of Helicobacter pylori infection as a risk factor. This bacterium, which was identified in gastric epithelium in 1984, has been extensively studied with regard to the risk of both esophageal and gastric AC. Most research suggests that it may protect against esophageal AC, but other studies have shown no such association. High intake of fruit and vegetables seems to reduce the risk, while a diet high in total fat or rich in saturated fat and cholesterol increases the risk of esophageal AC. It is being debated whether the use of medication that relaxes the lower esophageal sphincter, such as nitroglycerines, anticholinergics, β-adrenergic agonists, aminophyllines, benzodiazepines, calcium channel blockers, and tricyclic antidepressants, increase the risk of esophageal AC. Non-steroidal anti-inflammatory drugs (NSAIDs) might decrease the risk of developing this cancer, but this possibility has been questioned, as confounding by indication might be a factor underlying this result and thus further epidemiological studies are warranted. Living without a partner more than doubles the risk, as does a low socioeconomic status.

The sex ratio

One of the most intriguing risk factors for developing esophageal adenocarcinoma is the male gender. There is a strong male predominance in the incidence of this disease worldwide, with a ratio of 6-8:1 in most Western societies. This ratio cannot be attributed to differences in the prevalence of known risk factors between the sexes. The hypothesis that female sex hormones, i.e. estrogen, may have a protective role against the development of this cancer has been proposed and is evaluated and discussed more extensively in this thesis.
Gastric Adenocarcinoma

Incidence and trends
Gastric cancer is the fourth most common cancer worldwide. In 2002 more than 930 000 cases were diagnosed. With a high death rate (700 000 per year), gastric cancer is the second most common cause of cancer death, after lung cancer.\(^1\) In Sweden it is a less common cancer; the number of new cases diagnosed in 2005 was 913, with 576 (63%) occurring in men and 337 in women.\(^2\)

More than 90% of all gastric cancers are adenocarcinomas, the remainder being mainly non-Hodgkin’s lymphomas or leiomyosarcomas.\(^63\) There are two types of gastric AC: intestinal and diffuse, according to Laurén’s classification.\(^64\) The intestinal type is characterized by the gland-like appearance of neoplastic cells (Illustration 1), while in the diffuse type, as the name implies, the cells lack cohesion and infiltrate the stomach wall without forming a distinct mass (Illustration 2).\(^64\) The intestinal type occurs more often in older patients\(^65-67\) and in men,\(^65, 67, 68\) affecting the distal part of the stomach.\(^69\) In contrast, the diffuse type is a feature of young patients,\(^67-69\) while men and women are equally affected,\(^66, 69\) and it is found more often in the corpus and fundus of the stomach.\(^69\) This type has been more closely associated with heredity, while the intestinal type is thought to be preceded by precancerous conditions such as atrophic gastritis and intestinal metaplasia.\(^69\)

While the incidence of gastric cancer is declining worldwide\(^70\) including in Sweden\(^71, 72\) (Fig. 2), the incidence of cardia gastric cancer has increased\(^8, 15, 19, 31, 73\) (Fig. 3). It has been debated whether this increase is real. As a possible result of misclassification the true incidence rate of cardia gastric AC could be either 45% higher or 15% lower than that reported in the Swedish Cancer Register.\(^32\) However, in a recent study it was found that the incidence of this tumor is similar to that reported in the Register.\(^40\)

![Incidence of gastric adenocarcinoma in Sweden 1970-2005](image)

**Figure 2.** The incidence of gastric adenocarcinoma in Sweden between 1970 and 2005. Incidence per 100 000 persons and year, stratified by sex.\(^24\)
Illustration 1
Gastric adenocarcinoma of the intestinal histological type in a 79-year-old woman (hematoxylin, 20x).

Illustration 2
Gastric adenocarcinoma of the diffuse type in a 41-year-old man. Signet-ring cells, a characteristic feature of diffuse cancer, are shown (arrows) (hematoxylin, 40x).

Prognosis
Although the prognosis in gastric cancer is poor, it is better than that of esophageal cancer. In Japan, where mass screening has been practiced since the 1960s, the 5-year survival rate is 53%, while in North America and Europe it is around 25%. Studies focused on the prognosis of gastric cancer...
do not distinguish between adenocarcinomas and other histological types. Since more than 90% of gastric cancers are adenocarcinomas, however, it may be assumed that the results of these reports also reflect the prognosis of AC. With regard to the specific localization of cancer in the stomach, in many reports a distinction between cardia and non-cardia gastric cancer is made. Cancer of the cardia has a much poorer prognosis.74

Etiology
The etiology of gastric cancer is multifactorial. The association between H. pylori infection and the development of gastric cancer is, however, well established74 and has been demonstrated in studies using cross-sectional,75 case-control,76 and prospective designs77 in various populations of the world. As the decreasing prevalence of H. pylori infection parallels the falling incidence of non-cardia gastric cancer, it has been hypothesized that this infection is a risk factor limited to the non-cardia cancer, and not of cardia cancer, since the incidence of cardia cancer is increasing (Fig. 3). It has even been suggested that H. pylori infection has a protective effect against cardia cancer. A meta-analysis based on prospective cohort studies showed that the infection is associated with a risk of developing non-cardia gastric cancer but not cancer of the cardia.78 This has also been verified in a Norwegian study.79 In addition, a lower prevalence of this infection has been reported in patients with tumors of the cardia compared to those with tumors in the antrum of the stomach.80 The largest study, however, reported no association between H. pylori infection and cardia adenocarcinoma53. Thus, whether eradication of this infection leads to an increase risk of cardia gastric cancer is debatable. Numerous studies have shown a protective effect against gastric cancer of diets rich in fruit and vegetables,81 which has been confirmed in a Swedish population-based case-control study.82 Intake of salt seems to possibly increase the risk of this cancer.74 Tobacco has recently been established as a risk factor,83 and various groups, among them groups in Scandinavia, have reported an increased risk of gastric cancer in connection with tobacco smoking, while no association with alcohol drinking has been found.41, 48, 84, 85 Obesity is another risk factor for developing cancer of the cardia, but not of non-cardia gastric cancer.41, 43, 45, 86 A higher socioeconomic status has

![Incidence of cardia gastric adenocarcinoma in Sweden 1970-2005](image)

**Figure 3.** The incidence of cardia gastric adenocarcinoma in Sweden between 1970 and 2005. Incidence per 100 000 persons, stratified by sex. 24
been found to be associated with a reduced risk of gastric AC, and that risk was stronger for the cardia site or the intestinal histological type.\textsuperscript{87}

\textbf{The sex ratio}

As with the incidence of esophageal AC, there is a clear male dominance in the incidence of gastric AC, although less strong. In the Western societies the male-to-female ratio is about 2:1,\textsuperscript{4} which is similar to the sex ratio of gastric AC in Sweden. The ratio is higher for cardia gastric cancer (Fig. 3). For a worldwide perspective, summarized data are given in the Cancer Incidence in Five Continents, Volume 7.\textsuperscript{88} In that report, data from the National Cancer Registers of 50 countries are presented, and the report contains age-specific and country-specific incidence rates for major cancers in 183 populations. Registers which do not meet the high standards for quality and data compatibility are excluded. From these data, it is concluded that the male-to-female incidence ratio for cardia gastric AC is as high as that of esophageal adenocarcinoma in all countries studied.\textsuperscript{62} Data from the latest published volume of Cancer Incidence in Five Continents, in which 57 countries are included,\textsuperscript{89} again support the male dominance in esophageal and gastric cancer (although the data are not yet analyzed specifically for esophageal AC). As already mentioned, data from Sweden support these findings.\textsuperscript{2, 24, 31}
Estrogen in the Development of Esophageal and Gastric Adenocarcinoma
The Hypothesis

Various reports from different parts of the world have demonstrated the enigmatic male predominance in the incidence of esophageal and gastric adenocarcinoma. Since this sex ratio cannot be explained by sex differences in the prevalence of known risk factors, there should be endogenous factors that either provide protection in women alone, or imply an increased risk in men, but not in women. In the research presented in this thesis, it is hypothesized that women, as a result of their endogenous estrogen exposure, are protected from developing esophageal and gastric adenocarcinoma. Various studies offer some support for this hypothesis. An example with regard to the sex ratio of gastric AC is the global finding that women develop the intestinal type of gastric AC 10-15 years later than men and that the incidence of this type of AC increases after the menopause. That finding was consistent in all the 18 cancer registries used in the study. The potentially protective effect of estrogen against gastrointestinal cancer has been studied with respect to colorectal cancer. An ambitious study, the Women’s Health Initiative (WHI), in which more than 160 000 postmenopausal women enrolled between 1993 and 1998, focused on defining risks and benefits of strategies that could potentially reduce the incidence of breast and colorectal cancer, and of fractures in postmenopausal women. In that cohort, 16 608 women were randomized to either receive hormone replacement therapy (HRT) with estrogen and progesterin, or placebo. The trial was stopped after 5.2 years of follow-up because of an increased risk of invasive breast cancer in the HRT group, but in that group the risk of colorectal cancer was almost half of that of the non-HRT group (hazard ratio 0.56, confidence interval (CI) 0.38-0.81). Moreover, a meta-analysis of 18 observational studies showed a 20% reduction in the risk of colon cancer among women who had ever used HRT (relative risk 0.80, CI 0.74-0.86). Thus the notion of estrogen protection against gastrointestinal cancer is not new.
Estrogen

Production of estrogen
The primary estrogen in women is 17β-estradiol (E$_2$), which is produced in the granulosa cells of the ovaries. The estrogens estrone (E$_1$) and estradiol (E$_2$) are formed from estradiol in the liver. Other tissues in the body produce E$_2$ in addition (muscle, fat, nervous tissue), and E$_2$ has also been found in the testis. During menstrual cycles, the highest concentration of E$_2$ is in the preovulatory phase, while it is lowest premenstrually. In pregnancy, E$_1$ is synthesized in the placenta. In perimenopausal women, E$_2$ production declines, but the serum concentrations vary significantly. After the menopause the predominant estrogen is E$_1$ and the estrogen production in extragonadal tissue increases with increasing age and body weight.

Pathophysiological actions of estrogen
E$_2$ has multiple effects in the body. It stimulates growth in sexual organs, affects mood by regulating serotonergic, dopaminergic, and cholinergic neurons, and may explain the male dominance in the incidence of Parkinson’s disease. It reduces the risk of fractures in postmenopausal women who use HRT, it stimulates the uptake of low-density-lipoprotein cholesterol in the liver, and it decreases the intraocular pressure. When applied locally, estrogen reduces the depth of wrinkles and increases turgor. In addition it decreases the risk of colon cancer and in combination with progestin it decreases the risk of colorectal cancer. On the other hand, estrogen increases the risk of breast cancer, endometrial cancer, and venous thromboembolism. The potentially cardiovascular protective effects of estrogen have been discussed. Three large randomized control trials, among them the Women’s Health Initiative described above (see The hypothesis) found no evidence that HRT (estrogen plus progestin) was effective in preventing non-fatal myocardial infarction and deaths from coronary heart disease. Neither does it seem to protect against stroke – actually it may rather increase the risk of stroke. However, in the Nurse’s Health Study, a prospective observational cohort study in which more than 70 000 postmenopausal women were followed up for 20 years, estrogen reduced the risk of coronary events by almost 50% compared to never-users. Thus, the issue of cardiovascular protection remains controversial.

Molecular actions of estrogen
The most potent estrogen is E$_2$. Estrogen receptors (ERs), which belong to the nuclear receptor family of transcription factors, are attached to their receptor-associated proteins in the cytoplasm or the nucleus of the cell. The effects of estrogens in the cells are mediated through binding to these receptors. There are several mechanisms whereby this binding takes place. Estrogen can bind directly to ERs (the classical pathway), which then bind to DNA on estrogen response elements and thereby modulate gene regulation. In the tethered pathway, after

Nature does nothing uselessly.
Aristotle
activation of ERs following binding of estrogen, the estrogen-ER complex binds to proteins (other transcription factors), which in turn bind to DNA (indirect DNA binding). A third mechanism involves activation of ERs, which leads to a rapid physiological response (such as activation of ion channels) without involving gene regulation (non-nuclear actions). A fourth mechanism, the ligand-independent pathway involves activation of ERs independently of estrogen; for example by growth factors, which activate the ER, thereby binding DNA and regulating gene transcription.

**Estrogen receptors in esophageal and gastric adenocarcinoma**

It is proposed that the potential protective effect of estrogen against esophageal and gastric AC is exerted through ERs. Two types of ER are known: ER alpha (ERα) and ER beta (ERβ), both of which have been identified in esophageal AC and in Barrett’s esophagus. A plethora of studies have been published regarding the presence of ERs in non-cancerous and cancerous gastric tissue. Two years after the first report on ERβ, a splice variant of ERβ, termed ERβcx, was characterized. ERβcx is expressed in the breast, the prostate, the testis, and the esophagus, and we recently identified it for the first time in gastric tissue (papers III and IV).

**Selective estrogen receptor modulators (SERMs)**

Whereas the different types of estrogen are purely agonists, and antiestrogens are antagonists, selective estrogen receptor modulators (SERMs) exert both of these actions. SERMs belong to a family of chemically diverse compounds which although they lack the steroid structure of estrogen, can bind to ERs. The most commonly known SERM is tamoxifen, an antiproliferative agent used in the treatment of breast cancer. Tamoxifen gradually came into clinical practice during the late 1970s. It reduces the risk of recurrence and death, and effectively palliates metastatic breast cancer. Whether a SERM has an agonistic or an antagonistic effect depends on the different ERs expressed in a cell, the differential ER conformation on ligand binding, and the differential expression and binding to ER of coregulator proteins that either promote agonistic (coactivators) or antagonistic (corepressors) activity. Thus when acting in the breast, tamoxifen has an antagonistic effect, while when acting in the uterus it has an agonistic effect, increasing the risk of endometrial carcinoma. These two effects are considered to be mediated by the action of different coregulators (corepressors and coactivators) acting on ERα.
Aims of the studies

The overall aim of this thesis was to clarify the question whether estrogen protects women against the development of esophageal or gastric adenocarcinoma and thus explain the male dominance in the incidence of these tumors.

The specific aims were:

- To determine whether hormone replacement therapy protects postmenopausal women against esophageal or gastric adenocarcinoma (Paper I).

- To determine whether the use of tamoxifen increases the risk of esophageal or gastric adenocarcinoma (Paper II).

- To establish whether high endogenous estrogen exposure decreases the risk of the intestinal type of gastric adenocarcinoma, and whether such a potential decrease is mediated by estrogen receptors (Paper III).

- To determine whether use of tamoxifen entails an increased risk of the intestinal type of gastric adenocarcinoma, and whether such a potential effect is mediated by estrogen receptors (Paper IV).
Material and Methods

No great thing is created suddenly.
Epictetus

Table 1 gives an overview of the material and methods and of the outcomes analyzed in the four studies presented in this thesis. In the first two studies, large cohorts were followed up, and prospectively recorded data were obtained. In study I, the General Practice Research Database (GPRD) in the United Kingdom was used. In studies II-IV we collected data partly from the Swedish Cancer Register. In the last two studies additional data were gathered from medical records (study IV), a review of histological specimens, and immunohistochemical analysis of ERs (studies III & IV).

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GPRD: General Practice Research Database, HRT: hormone replacement therapy, BMI: body mass index, GI: gastrointestinal.
The General Practice Research Database (study I)

In the first study we used a large British database, namely the General Practice Research Database (GPRD) which collects data from general practitioners’ (GPs’) offices for research purposes. Since health care in the UK is centered around the general practitioner, most medical data of the patients are available in the medical records of the GPs. The GPRD started in the late 1980s, when a commercial company began to install computer systems in GPs’ offices across the UK. It was already expected at that time that data would be used in research, and after a trial period of data entry and quality training, more GP practices started to participate. Today more than 3 million people are covered by the participating GPs. A limited number of practices have provided prospective data to the GPRD since as early as 1987, but most of the practices have contributed since 1991. The size of the GPRD makes it efficient in the study of rare diseases. Validation studies have shown that this database maintains a high standard of data quality, with near-complete medical data in the GPs’ computers. The computerized information in the database includes: demographics, diagnoses, details of every consultation with general practitioners, a summary of specialists’ clinical notes and hospital letters, results of laboratory tests and a free-text section. The most valuable sources of data in the GPRD are the prescriptions of drugs. Every prescription is directly generated from the GP’s computer, thus ensuring a complete and unselected recording of prescriptions, including the dosage instructions. Drugs are coded on the basis of a drug dictionary from the Prescription Pricing Authority, and diagnoses are coded by a modification of the Oxford Medical Information System. All this information is recorded prospectively.

The Swedish Cancer Register (studies II-IV)

The Swedish Cancer Register was established in 1958 for research purposes and it became nationwide in 1961. Clinicians and pathologists in Sweden are required to report all cancer cases to the Register. In addition to the cancer diagnoses, the Register contains information on sex, date of birth, date of diagnosis, codes for specific site and histology of cancers, and hospital codes. The patients are identified by a unique 10-digit National Registration Number which is given to each Swedish resident at birth or immigration. This identifier makes linkage between various registers possible. Emigrations and deaths can be identified through linkage to the Swedish Registers of Causes of Death and Emigration. Validation studies have shown that the Swedish Cancer Register has a completeness rate of 98% for both esophageal and gastric cancer diagnoses. Virtually all cancer cases (99%) are cytologically or histologically verified. For the registration of cancer diagnoses, the Register used the 7th revision of the International Classification of Diseases (ICD) (WHO/HS/CANC/24.1 Code for Anatomical Location) between 1958 and 1986. For the years 1987-1992, the ICD-9 coding system was followed (WHO 9th revision 1976), and for the period 1993-2004 the ICD-O-2/ICD-10 (International Classification of Diseases for Oncology. Second Edition. WHO Geneva 1990) system. Since 2005 the ICD-O/3 (3rd Edition. WHO Geneva 2000) has been used (Table 2). ICD-O is a dual classification with coding systems for both the topography and morphology of the tumor. The topography code describes the site of origin of the tumor and uses the same 3-character and 4-character categories as in the neoplasm section of Chapter II, ICD-10. The morphology code describes the characteristics of the tumor itself, including the cell type and biological activity. The Swedish Cancer Register translates the different classifications to ICD-7 to enable longer trends to be identified.

Study I

Study cohort, participants, and exposure

This was a case-control study nested within the GPRD during the period January 1st, 1994 through December 31st, 2001. All women between the
Of ages 50-84 years recorded in the GPRD were identified and were eligible for inclusion in the study if they had been enrolled with a GP for at least 2 years, and had at least one year of computerized prescription history. Women with a cancer diagnosis recorded before the start of the study period were excluded. All participants were followed up until the first occurrence of esophageal or gastric cancer, detection of any other cancer, an age of 85 years, death, or the end of the study period. The computerized medical records of the patients (cases) with esophageal or gastric cancer were manually reviewed without knowledge of whether they had received HRT or not. A patient with this diagnosis was excluded from the study if: 1) the tumor was benign, 2) the origin of the cancer was unknown, 3) the tumor was a metastasis, 4) the patient had another concurrent cancer, 5) the cancer was diagnosed before the starting date of the study, or 6) the tumor was not AC or SCC.

The index date for each of our cases was the date on which their cancer was first diagnosed. For our potential controls the index date was set as follows: First all persons received a random date within the study period, and if this date was within that individual’s eligible person-time, she was marked as an eligible control. Thereafter, we randomly selected controls, matched for age and calendar year, and their random dates were set as index dates. Only use of HRT prior to the index date was considered. HRT included oral estrogen, transdermal estradiol, estradiol implants, and triboleone, which is a commonly prescribed steroid in Europe; a form of synthetic HRT. The use was classified as non-use if no recorded prescription was found, or as ever use if there was any such use. Users of HRT were further categorized into current or past users. The duration of HRT use was calculated by summing the periods of consecutive prescriptions among ever users, and was grouped into less than three years or 3 years and more. We identified 705 women with esophageal or gastric cancer and after exclusion of 93 cases that were not eligible, there remained 299 cases of esophageal cancer and 313 of gastric adenocarcinoma.

**Statistical analysis**
We calculated odds ratios (ORs) with 95% CI by unconditional logistic regression. In multivariable analyses we adjusted for various potential confounding factors.

### Study II
**Study cohort, participants, and exposure**
This was a population-based cohort study in which data from the Swedish Cancer Register were retrieved. All postmenopausal women of ≥50 years...
of age with a first, primary breast cancer diagnosed between January 1st, 1961 through December 31st, 2003 were included. Cross-linkage within the Cancer Register identified all study participants who during the study period developed an esophageal or gastric AC. To adjust for possible confounding by smoking, we also analyzed the risk of tobacco-related cancers, i.e. esophageal SCC and lung cancer. Since the Cancer Register has distinguished between cardia and non-cardia gastric cancer since the year 1970, the follow-up period with regard to the risk of gastric cancer started that year. Women diagnosed with a breast cancer before 1988 were considered not to have received tamoxifen treatment, whereas those diagnosed in 1988 and thereafter were considered to have been exposed to tamoxifen, since widespread use of tamoxifen in Sweden began only in the late 1980s. Among 138 885 postmenopausal women with breast cancer contributing with a total of more than 1 million person-years at risk, we identified 19 women with esophageal AC, 21 with cardia gastric AC and 341 with non-cardia gastric AC.

Statistical analysis
We estimated relative risks by calculating the standardized incidence ratio (SIR). This is the ratio of the observed to the expected number of newly diagnosed cancer cases. To minimize surveillance bias, women with an esophageal and gastric cancer diagnosis occurring within one year of their breast cancer were excluded.

Study III
Study cohort and participants
In the third study, we identified patients diagnosed with gastric cancer in the county of Stockholm during the period 1958-2004. They were categorized into three groups with regard to their endogenous estrogen exposure at the time of the diagnosis: 1) females younger than 50 years of age, representing the group of “exposed women”, 2) males younger than 50 years, representing the group of “unexposed men”, and 3) females over the age of 70 years, representing the group of “unexposed women”. The Register identified 364 cases in the first group, 396 in the second and 3008 in the third. We selected the latest 150 cases in each group (n=450) and requested histological sections from biopsies and surgical specimens of the cases filed at the six major pathology departments in the county of Stockholm (n=416). These were the Departments of Pathology at Karolinska University Hospital in Solna and Huddinge, St. Göran Hospital, Stockholm South General Hospital (Södersjukhuset), Danderyd Hospital, and Medilab AB, a private laboratory center. Of the 416 requested sections, we received 356 (86%), and verified gastric AC in 289 patients. Each gastric AC was classified into the intestinal or diffuse type in accordance with the Laurén classification. Specimens of mixed histological types were classified in conformity with Ming as being of the diffuse type. We performed immunohistochemistry, according to the protocol described on the next page, on sections from 45 cases with intestinal gastric AC. We then evaluated each section for the presence of ERα, β, and βcx, both in gastric cancer tissue and in the adjacent non-tumor tissue, if available in the section. Classification of the histology of the gastric AC and immunohistochemical analyses and assessment of the specimens with regard to the presence of ERs were done blindly, i.e. without knowledge of the sex and age of each patient.

Study IV
Study cohort, participants, and exposure
In the last study, we again first identified case participants from the Swedish Cancer Register. We identified 211 women in the county of Stockholm who were first diagnosed with breast cancer and who subsequently developed gastric cancer in 1958-2005. We collected the medical records of 107 (51%) and reviewed these to verify the diagnoses and the dates, and to classify each patient with regard to tamoxifen treatment. We had complete data for 101 patients. Of these, eight were excluded because their gastric cancer diagnosis
occurred within one year after the breast cancer. This exclusion was made to avoid surveillance bias; that is patients with a newly diagnosed cancer would be more likely to have another cancer detected because of the ongoing diagnostic examinations. Of the remaining 93 cases, we obtained histological material for 76 and evaluated their tumor histology. Thereafter, 68 with gastric AC were included in our final analyses and categorized according to Laurén’s classification into the intestinal or diffuse type. Immunohistochemistry was conducted according to the protocol described below to estimate the expression of ERs. Evaluation regarding the histological type of the gastric AC, and immunohistochemistry were done blind with respect to tamoxifen exposure.

**Statistical analysis (studies III & IV)**

By logistic regression, relative risks expressed as ORs with 95% CI were estimated. Fisher’s exact test was used to compare the frequency distributions of the three types of ER among the groups.

**Immunohistochemistry and evaluation of estrogen receptors (studies III & IV)**

Since the specimens from the selected tumors were mainly endoscopic biopsy samples, only a limited amount of tissue was available for immunohistochemistry in some cases. Consecutive sections were cut from paraffin-embedded material and immunohistochemistry was performed. We used a commercially available antibody for ERα, while the antibodies for detection of ERβ and ERβcx came from Professor Jan-Åke Gustafsson’s laboratory at Novum in Karolinska Institutet. The following protocol was used: The 4 µm thick paraffin sections were dewaxed in xylene and rehydrated through graded ethanol to water. Antigens were retrieved in 10 mMole citrate buffer and boiled for 30 minutes. Cooled sections were incubated in methanol containing 0.3% hydrogen peroxide for 30 minutes to block endogenous peroxidase. Thereafter, they were incubated in phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA) and 0.5% Nonidet P-40 for 10 minutes at room temperature to block non-specific binding. The sections were then incubated with the following antibodies in the given dilutions: Rabbit anti-ERα (1:120), chicken polyclonal antibody anti-ERβ 503 IgY (1:200) and anti-ERβcx sheep polyclonal antibody (1:200). Human breast tissue was obtained for positive control. Sections were incubated in 5% BSA in PBS overnight at room temperature. Secondary antibodies in PBS were applied for 60 minutes at room temperature, after which the slides were rinsed with PBS and then incubated for 60 minutes with avidin biotin complex. They were further washed with PBS and colored with 3, 3’-diaminobenzidine tetrahydrochloride substrate. The sections were finally counterstained with Mayer’s hematoxylin and dehydrated through graded ethanol series to xylene, and mounted with pertex.

Immunohistochemical evaluation was performed according to the recommendation of Wang, Mangano and Antonioli, according to which areas with the highest degree of expression should be evaluated. There are two reasons for this: First, the number of positive cells to be counted may vary from one field to another and the average count might not sufficiently reflect the severity of changes in the entire biopsy. The second reason is the varying size of the biopsy samples, many of which may have contained less than three well-oriented high power fields. In that case the average count would have been derived from a varying number of fields.

Specimens with 50% or more of the cells, in a high power field, positive for ERs were considered positive. ERs are mainly found in the nucleus, but immunoreactivity in the cytoplasm has also been shown. In our studies, sections with positive immunoreactivity in the nucleus were classified in the nucleus-positive group, while those with exclusively cytoplasmic expression were assigned to the non-nuclear-staining group. We examined both the gastric cancer tissue and the adjacent non-tumor tissue.
Estrogen in the Development of Esophageal and Gastric Adenocarcinoma
Results

Big results require big ambitions.

Heraclitus

Study I

Hormone replacement therapy and esophageal adenocarcinoma

The results concerning the association between HRT with estrogen and risk of esophageal cancer are presented in Table 3. The risk of esophageal cancer of any histological type was not statistically significantly related to use of HRT (OR 0.84, 95% CI 0.51-1.38). The odds ratio for esophageal AC among HRT users was not decreased (OR 1.17, 95% CI 0.41-3.32). Owing to the limited number of exposed cases, we could not perform valid analyses of use duration or recency among users of HRT. The point estimates were similar, however, regarding treatment duration, while lower point estimates were found among current users (OR 0.68).

Study II

Hormone replacement therapy and gastric adenocarcinoma

Table 4 shows the association between HRT and gastric adenocarcinoma. The risk of gastric AC was reduced by more than 50% (OR 0.48, 95% CI 0.29-0.79) among ever users of HRT compared to never users. The risk estimates for cardia and non-cardia gastric AC were both lower among women with HRT, but the decrease was greater and statistically significant only for non-cardia gastric cancer (OR 0.34, 95% CI 0.14-0.78). Stratified analysis by age group revealed the lowest risk among women older than 60 years (OR 0.38, 95% CI 0.18-0.84). We did not find any differences between smokers and non-smokers with regard to their risk of gastric AC. Current users of HRT had a lower risk (OR 0.56, 95% CI 0.33-0.96) and this risk was further decreased among past users (OR 0.25, 95% CI 0.09-0.70). No dose-response effect was found regarding duration of HRT use.

Tamoxifen and esophageal adenocarcinoma

In the tamoxifen-exposed cohort we found 9 women with esophageal AC, contributing to a 60% statistically non-significant increase in the risk of developing this tumor (SIR 1.60, 95% CI 0.83-3.08). The risk decreased with increasing latency interval after the breast cancer diagnosis, however. In the unexposed cohort the risk for the same tumor was increased by 17% (95% CI 0.63-2.18) and no trend was observed with increasing latency interval (see Table 1, paper II).

Tamoxifen and gastric adenocarcinoma

We identified 21 cases of cardia gastric AC in the total cohort. The risk of this tumor was not increased in either the exposed or the unexposed cohort. Nor did we find any increase in the risk with increasing latency interval after the breast cancer diagnosis (Table 2, paper II). The number of non-cardia gastric AC cases in the total cohort was 341, which meant an overall increase in the risk of this cancer (SIR 1.41, 96% CI 1.27-1.57) (Table 5). In the tamoxifen-exposed cohort, the observed increase in risk was statistically significant (SIR 1.27, 95% CI 1.03-1.57) and it increased with an increasing latency.
Table 3. Odds ratios (ORs) with 95% confidence intervals (CIs) for the association between use of hormone replacement therapy (HRT) and risk of esophageal cancer.

<table>
<thead>
<tr>
<th>HRT</th>
<th>Control subjects</th>
<th>Esophageal adenocarcinoma</th>
<th>Esophageal squamous cell carcinoma</th>
<th>Esophageal cancer of unknown histology</th>
<th>Total esophageal cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
</tr>
<tr>
<td>Never</td>
<td>2,846 (89.2)</td>
<td>53 (91.4) 1.00 (reference)</td>
<td>66 (89.2) 1.00 (reference)</td>
<td>157 (94.0) 1.00 (reference)</td>
<td>276 (92.3) 1.00 (reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>345 (10.8)</td>
<td>5 (8.6) 1.27 (0.46 – 3.48)*</td>
<td>8 (10.8) 0.94 (0.42 – 2.15)*</td>
<td>10 (6.0) 0.56 (0.28 – 1.15)*</td>
<td>23 (7.7) 0.78 (0.48 – 1.26)*</td>
</tr>
</tbody>
</table>

*ORs adjusted only for age. †ORs adjusted for age, calendar year, tobacco smoking, alcohol consumption, body mass index, hysterectomy, and upper gastrointestinal disorders.

Table 4. Odds ratios (ORs) with 95% confidence intervals (CIs) for the association between use of hormone replacement therapy (HRT) and risk of gastric adenocarcinoma.

<table>
<thead>
<tr>
<th>HRT</th>
<th>Control subjects</th>
<th>Cardia gastric adenocarcinoma</th>
<th>Non-cardia gastric adenocarcinoma</th>
<th>Unknown site of gastric adenocarcinoma</th>
<th>Total gastric adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
<td>N (%) OR (95% CI)</td>
</tr>
<tr>
<td>Never</td>
<td>2,846 (89.2)</td>
<td>38 (88.4) 1.00 (reference)</td>
<td>109 (94.0) 1.00 (reference)</td>
<td>143 (92.9) 1.00 (reference)</td>
<td>290 (92.6) 1.00 (reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>345 (10.8)</td>
<td>5 (11.6) 0.85 (0.30 – 2.44)*</td>
<td>7 (6.0) 0.34 (0.15 – 0.77)*</td>
<td>11 (7.1) 0.61 (0.30 – 1.21)*</td>
<td>23 (7.4) 0.51 (0.32 – 0.83)*</td>
</tr>
</tbody>
</table>

*ORs adjusted for age. †ORs adjusted for age, calendar year, tobacco smoking, alcohol consumption, body mass index, hysterectomy, and upper gastrointestinal disorders.
interval after the breast cancer diagnosis. In the unexposed cohort there was again a statistically significantly increased risk of non-cardia AC (SIR 1.47, 95% CI 1.30-1.66), but with an increasing latency interval the risk decreased. In the analyses of all gastric cancers, including tumors without specification of histological type or gastric sub-site (n=598), gastric AC without site-specific information (n=503), and non-cardia gastric cancer without histological specification (n=405), the risk estimates were all generally similar to those presented for tumors specified as non-cardia gastric adenocarcinomas (data not shown).

Tamoxifen and esophageal squamous cell carcinoma and lung cancer
In the cohort unexposed to tamoxifen, there was an overall increase in the risk of esophageal squamous cell carcinoma (SIR 1.56, 95% CI 1.21-2.02) and also a slight increased risk of lung cancer (1.10, 95% CI 0.99-1.22). In the exposed cohort no such increased risks were found (0.99, 95% CI 0.59-1.64 and 0.84, 95% CI 0.73-0.97, respectively).

Table 5. Characteristics of a cohort of postmenopausal Swedish women with a breast cancer diagnosis between 1970 and 2003, and the standardized incidence ratio (SIR), with 95% confidence interval (CI), of non-cardia gastric adenocarcinoma. Patients diagnosed between 1970 and 1987 were considered unexposed to tamoxifen treatment, while those diagnosed between 1988 and 2003 were considered to be exposed. The first year of follow-up was excluded. The earliest data for the gastric cancer diagnosis were available from 1961, but distinction between cardia and non-cardia diagnoses was only made from 1970.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of breast cancer cases</td>
<td>53,643</td>
<td>65,505</td>
<td>119,148</td>
</tr>
<tr>
<td>No. of person-years at risk</td>
<td>528,789</td>
<td>363,765</td>
<td>892,555</td>
</tr>
<tr>
<td>Mean years of follow-up</td>
<td>9.85</td>
<td>5.55</td>
<td>7.49</td>
</tr>
<tr>
<td>Mean age at entry (range)</td>
<td>69 (50-101)</td>
<td>68 (50-104)</td>
<td>68 (50-104)</td>
</tr>
<tr>
<td>No. of observed non-cardia gastric adenocarcinoma cases</td>
<td>257</td>
<td>84</td>
<td>341</td>
</tr>
<tr>
<td>No. of expected non-cardia gastric adenocarcinoma cases</td>
<td>174.52</td>
<td>65.88</td>
<td>240.39</td>
</tr>
<tr>
<td>SIR (95% CI)</td>
<td>1.47 (1.30-1.66)</td>
<td>1.27 (1.03-1.57)</td>
<td>1.41 (1.27-1.57)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Latency interval after breast cancer diagnosis in years</th>
<th>Number of observed cases of non-cardia gastric adenocarcinoma, SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>105, 1.7 (1.4-2.06) 46, 1.29 (0.96-1.72) 151, 1.55 (1.32-1.82)</td>
</tr>
<tr>
<td>5-9</td>
<td>71, 1.4 (1.11-1.77) 24, 1.06 (0.71-1.58) 95, 1.3 (1.06-1.58)</td>
</tr>
<tr>
<td>10-14</td>
<td>39, 1.19 (0.87-1.63) 14, 1.86 (1.1-3.14) 53, 1.32 (1.008-1.72)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>42, 1.41 (1.04-1.91) 0 42, 1.4 (1.04-1.9)</td>
</tr>
</tbody>
</table>
Study III
Distribution of histological types of gastric adenocarcinoma
In 11% of the cases in the group of “exposed women” the gastric AC was of the intestinal type. In comparison with this group, the occurrence of the intestinal type was significantly more common among “unexposed men” (OR 4.7; 95% CI 2.2-10.3) and “unexposed women” (OR 9.1; 95% CI 4.3-19.6), respectively (Table 6).

Estrogen receptors
For the first time, the existence of ERβcx was discovered in gastric tissue. We found no differences between the three groups of patients, however, with regard to the expression of ERα, β, and βcx in the gastric AC tissue or in the adjacent non-tumor tissue. This lack of difference was true for both the nuclear and non-nuclear stainings. The only statistically significant difference was found in the expression of ERα in the AC tissue (non-nuclear staining), where fewer unexposed men had ERα compared to exposed women (for detailed tables please refer to Paper III). In all three groups of patients the frequency of positive ERα was higher in the AC tissue than in adjacent tissue. The opposite trend was noted for ERβ and βcx; that is, positive β and βcx receptors were less frequent or non-existent in the gastric AC tissue than in non-tumor tissue (Table 7). Examples of ER expression are shown in Illustration 3 (A-D).

<table>
<thead>
<tr>
<th>Estrogen exposure group</th>
<th>Intestinal type</th>
<th>Diffuse type</th>
<th>Total</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Exposed women</td>
<td>10 (11)</td>
<td>80 (89)</td>
<td>90</td>
<td>4.7 (2.2-10.3)</td>
</tr>
<tr>
<td>Unexposed men</td>
<td>35 (37)</td>
<td>59 (63)</td>
<td>94</td>
<td>1.0 (reference)</td>
</tr>
</tbody>
</table>

Table 7. Comparison of positive nuclear staining between adenocarcinoma and non-tumor, adjacent, gastric mucosa with regard to the presence of estrogen receptors (ER) α, β and βcx. Exposed women: <50 years of age; unexposed men: <50 years of age; unexposed women: >70 years of age (for the non-nuclear staining table, please refer to Paper III).

<table>
<thead>
<tr>
<th>Estrogen Exposure group</th>
<th>ERα+ (%)</th>
<th>ERβ+ (%)</th>
<th>ERβcx+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor</td>
<td>Non-tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>Exposed women</td>
<td>25</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Unexposed men</td>
<td>40</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>Unexposed women</td>
<td>23</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>
Study IV
Distribution of histological types of gastric adenocarcinoma
Table 8 shows the distribution of the histological types of gastric AC. No significant differences between the groups were found.

Estrogen receptors
There were no statistically significant differences in the expression of any of the three types of ER in the two groups, either in the gastric AC tissue or in the adjacent non-tumor tissue. This was the case for both the nuclear and non-nuclear staining. Examples of ER expression are shown in Illustration 3 (A-D).

Latency interval between breast cancer diagnosis and gastric adenocarcinoma
The mean age at the time of the breast cancer diagnosis was similar between the two groups. The mean interval between the breast and the gastric cancer diagnoses was shorter in those treated with tamoxifen (4 vs. 13 years). When we restricted our analysis to patients who received a breast cancer diagnosis in 1978 or later (the year in which the first tamoxifen treatment was registered in our cohort), a difference in latency remained. Breast cancer patients who had been exposed to tamoxifen were diagnosed with gastric cancer after 4 years, compared to 10 years in those with no such exposure (Table 9).

Table 8. Histological types of gastric adenocarcinoma, according to the Laurén classification, in women with previous breast cancer diagnosed in the county of Stockholm in 1958-2005. Tamoxifen exposure was categorized into use or non-use.

<table>
<thead>
<tr>
<th>Tamoxifen Treatment</th>
<th>Intestinal Number (%)</th>
<th>Diffuse Number (%)</th>
<th>Total Number (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>13 (34)</td>
<td>25 (66)</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (27)</td>
<td>22 (73)</td>
<td>30</td>
<td>1.43 (0.5-4.08)</td>
</tr>
<tr>
<td>Total</td>
<td>21 (31)</td>
<td>47 (69)</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Age at onset of breast cancer and of gastric adenocarcinoma among women diagnosed with both these tumors in the county of Stockholm in 1978-2005. The tamoxifen exposure was categorized into use or non-use.

<table>
<thead>
<tr>
<th>Tamoxifen</th>
<th>No tamoxifen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>30 (68)</td>
<td>14 (32)</td>
</tr>
<tr>
<td>Mean age at breast cancer diagnosis in years (range)</td>
<td>67 (42-87)</td>
<td>66 (56-79)</td>
</tr>
<tr>
<td>Mean age at gastric adenocarcinoma diagnosis in years (range)</td>
<td>72 (47-89)</td>
<td>77 (65-88)</td>
</tr>
<tr>
<td>Mean number of years between breast and gastric adenocarcinoma diagnoses in years (range)</td>
<td>All: 4 (2-9)</td>
<td>All: 10 (4-19)</td>
</tr>
<tr>
<td></td>
<td>Intestinal (n=8): 5 (2-7)</td>
<td>Intestinal (n=5):11 (6-19)</td>
</tr>
<tr>
<td></td>
<td>Diffuse (n=22): 4 (2-9)</td>
<td>Diffuse (n=9): 9 (4-16)</td>
</tr>
</tbody>
</table>
Illustration 3

A. Gastric adenocarcinoma of the intestinal type expressing nuclear estrogen receptor alpha in an 80-year-old woman with previous breast cancer. The patient had not been treated with tamoxifen.

B. Non-tumorous glands adjacent to gastric adenocarcinoma of the intestinal type (not shown) in a 79-years-old woman, expressing estrogen receptor beta in the cytoplasm. The patient had been treated with tamoxifen.

C. Non-tumorous glands adjacent to gastric adenocarcinoma of the intestinal type (not shown), in a 42-year-old woman, expressing estrogen receptor beta cx in the cytoplasm.

D. Pyloric glands adjacent to gastric adenocarcinoma of the intestinal type (not shown), in a 79-year-old woman, expressing estrogen receptor beta cx in supra-nuclear granules.
Discussion

The studies presented in this thesis have provided evidence in favor of the hypothesis that estrogen protects women against the development of gastric adenocarcinoma, but not esophageal adenocarcinoma. The correctness of these main findings will be discussed in the following.

Animal studies suggest that hormonal factors may play a suppressive role in the development of gastric cancer. The carcinogenic N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) added to drinking water of rats induced gastric cancer in male rats but not in female. In the same experiment, castrated or estrogen-treated male rats had a lower incidence of gastric cancer compared to untreated male rats. In addition, the incidence increased in castrated female rats. Administration of estrogen in previously MNNG-treated rats reversed MNNG-induced alterations. Moreover, administration of female sex hormones to male rats decreased their incidence of gastric cancer. On the other hand, estrogen effects on the growth of human gastric cancer xenografts in nude mice have been contradictory; some gastric cancers have been stimulated, while others have been inhibited or not affected at all.

The discovery of estrogen receptors in gastric tissue in 1983 ignited enormous interest in the possible implication of this finding for the treatment of gastric cancer. The potential role of involvement of hormonal factors was discussed and some groups suggested hormonal therapy with estrogen. Since then, a number of researchers, using various methods, have observed the presence of ERs in gastric tissue, either in the cytoplasm or in the nucleus. Some have reported on the ER status in association with clinicopathological data but these studies were too small for the findings to be applicable to clinical praxis.

In contrast to the large number of studies reporting on the presence of ERs in gastric tissue, very few groups have studied the presence of estrogen receptors in esophageal tissue. Laboratory research suggests that estrogen may have an inhibitory effect in esophageal cancer, both in vivo and in vitro. Nevertheless, there appear to have been no studies assessing the ER status in esophageal cancer in relation to clinicopathological features.

Although the hypothesis of a protective effect of estrogen was stronger for gastric AC compared to esophageal AC, we decided in the planning stage of this thesis to study both these tumors because of the existence of the enigmatic sex ratio in their incidence. First, to further provide evidence for or against the hypothesis, we conducted two large epidemiological studies to assess the risk of developing these two cancers in relation to hormone replacement therapy with estrogen (study I) and tamoxifen exposure (study II). In studies III and IV we concentrated our efforts on the positive findings in the first two studies with regard to gastric AC. The possible influences of endogenous estrogen and the anti-estrogen tamoxifen were evaluated by combining epidemiological data collection with histopathological and immu-
nrohistochemical laboratory work, in an effort to elucidate the question whether ERs can explain the sex ratio in gastric adenocarcinoma.

Before continuing with the discussion of this thesis, it is worth considering some basic epidemiological aspects. There are two types of epidemiological studies: Observational and experimental. Studies in which an intervention is tested (for example the evaluation of a new drug) are often experimental, and of these randomized controlled trials are most common in clinical studies. None of the studies described in this thesis were experimental, all being observational. In observational studies, nature is allowed to take its course and the effects of an exposure or treatment, for example, are followed up with regard to a specified outcome. An observational study can simply be a case report in which a clinical characteristic or outcome from a single clinical subject or event is reported, or a cross-sectional study in which the presence or absence of a disease together with other variables in a study population at a particular point in time is investigated. Another main type of study design is a case-control study, in which a group of people with the disease under study is identified and compared with a suitable comparison group without the disease. A case-control study can help to determine the causal relation between an exposure and a disease, and is usually most feasible for studies of diseases with a very low incidence or prevalence (e.g. esophageal AC in women, as in study I). A case-control study is typically retrospective regarding the exposure assessment; that is, the exposures of the study subjects are tracked backward in time. The special type of case-control study that is nested within a defined cohort, however, allows a prospective exposure assessment. The first study presented in this thesis had a nested case-control design; i.e., cases and controls were derived from the same cohort, in which exposure data were collected before the occurrence of the disease under study. Another main type of observational study is the cohort study. In contrast to a case-control study, in a cohort study the disease is the end-point and a group of persons with a known exposure status is followed up over time. Cohort studies can determine the incidence of a disease, and are particularly useful for assessing the effect of rare risk factors. Among the present investigations, study II was a cohort study in which the effect of tamoxifen (as a risk factor) in the two groups of patients (one in the period 1961-1987 and one in 1988-2003) was addressed. Although the groups were followed up prospectively in time, this was a retrospective study, since the outcome (gastric or esophageal AC) had already occurred when we started the investigation.

In epidemiological research any deviation from the truth is due to systematic error (bias) or random error. Systematic errors can be further classified into selection bias, information bias and confounding.

Selection bias (sampling bias) occurs when the sampling is not representative of the population. For instance, this often happens when people who choose to participate in the study are different in one or more respects from those who choose not to do so.

Information bias occurs when the information collected is distorted. This can be measurement bias, i.e., misclassification of the exposure or the disease. Recall bias is a common problem in case-control studies, when cases and controls recall events in the past differently depending on their health status. Experimenter expectancy (the Pygmalion effect) is a problem in experimental studies when the experimenter’s expectations are inadvertently communicated to subjects who then produced the desired results. Lead-time bias happens when a disease is diagnosed earlier than normally on account of the study, resulting in false estimation of, for example, survival rates. Late-look bias occurs when patients with a severe disease are less likely to be studied because they die first. Information bias can be differential (different between the comparison groups) or non-differential (the same degree of bias in the comparison groups). Differential bias can show false associations when there are none, or obliterate associations when they actually exist. Non-differential bias usually dilutes true associations.
against the null, and therefore cannot explain remaining associations.

**Confounding** occurs when, simply put, there is a confusion, or mixing, of effects. A confounding factor has three properties: a) it is associated with the disease (as a cause and not as an effect of it), b) it is associated with the exposure, and c) it is not an effect of the exposure. Some ways for controlling confounding are: *randomization* (subjects randomly allocated to different groups; this can only be used in experimental studies), *restriction* (limiting the study to a group of subjects with the same, or nearly the same, value of a factor that might be a confounder), *matching* (subjects of different groups are paired together with regard to a confounding factor), *stratification* (the exposure-disease analysis is done in different categories with regard to the occurrence of a confounder), and *multivariable regression analysis* (a mathematical model in which each confounder is analyzed one at a time). Systematic errors cannot be reduced by increasing the size of the study.

**Random errors** can be counteracted by increasing the sample size. In type I (α error) the null hypothesis is incorrectly rejected, i.e., a statistically significant effect is found even though there is none. In type II (β error) the null hypothesis is incorrectly not rejected, i.e., no effect is found even though there is one.

In the first two studies we used population-based cohorts. Both the General Practice Research Database and the Swedish Cancer Register have been shown to have a high quality of data, which are collected prospectively. In the first study HRT use was recorded before the outcome (esophageal or gastric AC), and in the second study the cohort was defined on the basis of the breast cancer diagnosis, which was also recorded before the end-point outcome (esophageal or gastric AC). In this way selection and information bias was avoided or at least reduced.

In study I, our manual review of all the cases further decreased the risk of information bias. The fact that we did not find any association between HRT and esophageal cancer indicates that selection bias should not have explained the inverse association found with gastric cancer. Our analyses were adjusted for a number of possible confounding factors (age, smoking, alcohol consumption, BMI), but the risk of confounding by known or unknown factors cannot be ruled out. Moreover, residual confounding by factors adjusted for cannot be excluded. An example of a potential confounder is socioeconomic status, since a low socioeconomic status has been associated with an increased risk of esophageal and gastric AC, and the use of HRT might be influenced by such status.

In the second study, tobacco smoking might have acted as a confounding factor in the tamoxifen-unexposed cohort. The risks of esophageal SCC and lung cancer, both of which are closely linked with smoking, were increased in the cohort unexposed to tamoxifen. Since smoking is also a risk factor for gastric AC, confounding by smoking could explain the increased risk of non-cardia gastric AC. With regard to exposure, we could not assess this individually for each cohort member. The cut-off year of 1988 was chosen for the reason that widespread tamoxifen treatment in Sweden started in the late 1980s. The use of a cut-off year means that misclassification of the exposure is unavoidable. Nevertheless, data from the county of Stockholm indicate that during the period 1989-1991 48% of all patients with invasive cancers received endocrine treatment (the most common of which being tamoxifen), and that since 1992 more than 90% have received such treatment. In addition, the exposure misclassification should be non-differential and thus should only dilute true associations and not explain the positive associations found in our study. The true association is likely, rather, to be stronger.

In the last two studies, we identified our study participants from the Swedish Cancer Register. In study III we were not able to adjust for potential confounding with regard to the distribution of histological types of gastric AC between the three patient groups. However, *H. pylori* infection should not affect the histological type. Neither should smoking or intake of alcohol. Fruits
or vegetables, or occupational exposure and these factors should therefore not have acted as confounders in our analysis of histological type. In the last study, the thorough review of medical records reduced the risk of information bias. Data on the tumor size, location, and gross appearance, invasive depth, invasion of lymph vessels or veins of the stomach wall, nodal involvement, peritoneal dissemination, liver metastasis, and curability were not available. But these clinicopathological characteristics do not influence the presence of ERs and would probably not have influenced our results of ERs in either of the last two studies. Moreover, the histological evaluation, the immunohistochemical laboratory work (studies III & IV), and the review of the medical records (study IV) were conducted blindly with regard to the estrogen exposure status of each patient or the tamoxifen exposure, diminishing the risk of systematic bias.

In the fourth study we analyzed the whole cohort of patients and found a decreased interval between the breast and gastric cancer diagnoses in those exposed to tamoxifen (4 vs. 13 years). This should, however, be interpreted with caution, as there is a risk for selection bias. Tamoxifen was introduced in the later half of the total follow-up period (1958-2005), and patients having that treatment who would have developed gastric cancer at a much later date (long latency interval) were not included in the analysis for the reason that the follow-up period in the study had ended. As a result, only those with a short latency interval would have been included in the analysis. However, stratified analysis showed that there was still a difference between the groups regarding the latency between these two cancers. The first breast cancer patient in our cohort who received tamoxifen treatment was registered in 1978. If only patients who were diagnosed with breast cancer in 1978 or later are analyzed, the latency interval in the tamoxifen group is 4 years, while that in the no-tamoxifen group is 10 years. Moreover, in study II we found that tamoxifen-exposed women had an almost doubled risk for non-cardia gastric AC after 10-14 years following the breast cancer diagnosis, compared to the general population. Thus, the results of these two studies indicate that tamoxifen increases the risk of gastric AC. Chance error was a major source of error in studies III and IV, particularly in the ER analyses, as a result of small sample sizes.

There have been reports on the risk of esophageal and gastric cancer in relation to hormonal factors, but only a few have specifically addressed the risk of esophageal or gastric AC. In our first study, we found a decreased risk of gastric AC in women who used HRT with estrogen. Previous studies concerning this association have also indicated a protective effect of HRT, with one exception. Table 10 presents an overview of epidemiological studies in which the risk of esophageal and/or gastric cancer was assessed. The majority of these studies were conducted on women, but in two studies the effect of estrogen in men was investigated. Patients with prostate cancer who were given estrogen showed a lower risk of developing gastric cancer than those with no such treatment, while no such association was found for esophageal AC. In our second study, we analyzed the effect of tamoxifen on the risk of esophageal and gastric AC. No effect regarding the incidence of esophageal cancer had been observed in the previous studies that had addressed tamoxifen as a risk factor. In a pooled analysis of three studies in Scandinavia a non-significant, but nearly 3-fold increase in the risk of gastric cancer was found, and correspondingly elevated risks have been reported from other studies. Our lack of a finding of any clear influence of anti-estrogen on the risk esophageal AC, but a positive association with gastric AC, is therefore in line with the majority of previous investigations. On the other hand, from two small but uncontrolled Japanese studies it was reported that patients with gastric cancer who were given tamoxifen had a survival advantage. In a randomized, controlled trial, 100 patients with gastric cancer who received conventional surgical management were assigned to either additional tamoxifen treatment
or to join an untreated control group. The conclusions drawn from that study were that tamoxifen had no overall effect on survival, and that there was a significant decrease in the survival time of patients with tumors positive for ERs.  

**Table 10. Epidemiological studies assessing the risk of esophageal and/or gastric cancer in relation to hormonal factors. Not all associations were significant.**

<table>
<thead>
<tr>
<th>Cancer studied</th>
<th>Age at menarche</th>
<th>Age at menopause</th>
<th>Length of fertility life</th>
<th>HRT</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB. Miller et al (Canada, 1980) 173</td>
<td>EC, GC</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>I. Plesko et al (Slovakia, 1985) 175</td>
<td>GC</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C. La Vecchia et al (Italy, 1993) 170</td>
<td>EC, GC</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>D. Palli et al (Italy, 1994) 174</td>
<td>GC</td>
<td>NA</td>
<td>+ lower age</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C. La Vecchia et al (Italy, 1994) 169</td>
<td>GC</td>
<td>NA</td>
<td>- higher age</td>
<td>- longer fertility life</td>
<td>- HRT, + OC</td>
</tr>
<tr>
<td>J. Lagergren et al (Sweden, 1998) 171</td>
<td>EAC</td>
<td>Estrogen in men did not affect the risk of EAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Heuch et al (Norway, 2000) 167</td>
<td>GC</td>
<td>- higher age</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S. Gallus (Italy &amp; Switzerland, 2001) 166</td>
<td>EC</td>
<td>Only squamous cell carcinoma cases were studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Inoue et al (Japan, 2002) 168</td>
<td>GC</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
</tr>
<tr>
<td>E. Fernandez et al (Italy, 2003) 163</td>
<td>GC</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Tend. to lower risk</td>
</tr>
<tr>
<td>S. Kaneko et al (Japan, 2003) 176</td>
<td>GC</td>
<td>NA</td>
<td>NA</td>
<td>- longer fertility life</td>
<td>- HRT</td>
</tr>
<tr>
<td>M. Lindblad et al (Sweden 2004) 172</td>
<td>GC</td>
<td>Reduced risk in men treated with estrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. Lagergren (Sweden 2005) 177</td>
<td>EAC</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S. Frise el al (Canada, 2006) 165</td>
<td>GAC</td>
<td>+ higher age</td>
<td>+ in post-menopause</td>
<td>- longer fertility life</td>
<td>- HRT or OC</td>
</tr>
<tr>
<td>ND. Freedman (China, 2007) 164</td>
<td>GC</td>
<td>NA</td>
<td>- higher age</td>
<td>+ shorter fertility life</td>
<td>NA</td>
</tr>
</tbody>
</table>

EC: esophageal cancer, GC: gastric cancer, EAC: esophageal adenocarcinoma, GAC: gastric adenocarcinoma, HRT: hormone replacement therapy, OC: oral contraceptives, NA: no association, NS: not studied, +: increased risk with, -: decreased risk with
In our studies III and IV, we analyzed specimens of estrogen receptors of only intestinal types of gastric AC and we categorized patients according to sex and age (study III) or tamoxifen exposure (study IV). This was a unique study design, as in previous studies specimens of gastric cancer were analyzed in groups categorized only with regard to age, or sex, or histological type, and never on the basis of various epidemiological and histological data. Our reason for the choice of the intestinal types was that epidemiological data had suggested that only this type of gastric AC may be affected by hormonal factors. A unique global pattern in the male to female ratio in the incidence of gastric AC has been reported, indicating that there is a 10-15-year delay among females compared to males, possibly due to a difference in estrogen exposure. This delay was exclusively seen for the intestinal type. In addition, in a recently published Canadian study, it was found that the association between age at menopause and risk for the intestinal type of gastric AC was highly significant, in contrast to the diffuse type. Although we did not find any differences between the three groups of patients with regard to their ERs, we did find a change in the expression of the ER types in gastric AC tissue in comparison with the adjacent non-tumor tissue. Expression of ERβ and βcx was decreased in gastric AC, while ERα expression was increased. This is consistent with reports on prostate, breast and colon cancer, and a recently published study has also confirmed the decreased expression of ERβ in gastric AC, compared to non-tumor tissue.

In study IV, we did not find that tamoxifen stimulates the progression of intestinal type adenocarcinoma exclusively. It is possible, however, that tamoxifen increases the overall risk of AC without affecting the histological type. This is supported by the fact that patients in the tamoxifen group may have a stronger heredity for cancer, as all of the nine patients with more than one breast cancer diagnosis had diffuse adenocarcinoma. As mentioned earlier the diffuse type has been postulated to be more likely to have a genetic origin, although this has been challenged.

In this thesis we found evidence to suggest that hormonal factors may play an important role in the development of gastric adenocarcinoma, while no support was found for a role in esophageal adenocarcinoma. The mechanisms, however, are still unclear.

It has been found that ovariectomy in rats increases the cell mass as well as basal acid secretion, suggesting that estrogen may regulate gastric acid production. Moreover, it has been hypothesized that bile acids may be carcinogenic, although this is debated. Estrogen may prevent colon cancer by decreasing the bile acid concentration or by direct effects on the colonic mucosa, as suggested by in vitro studies. This could explain why HRT with estrogen reduced the risk of non-cardia gastric adenocarcinoma, as this region of the stomach is exposed to bile acids to a greater extent. In addition, in breast cancer cells it has been found that bile acids down-regulate the expression of ERs. Another proposed mechanism is that estrogen affects the expression of trefoil factor (TTF) genes. TTF proteins protect mucous epithelia from a range of insults and contribute to mucosal repair. The expression of these genes is reduced in precancerous conditions and in gastric cancer, and estrogen has been found to stimulate their expression. Others suggest that estrogen may bind to ERs and inhibit the expression of c-erbB-2 oncogene or the expression of p185. The latter is associated with the progression of gastric cancer.

The exact action of tamoxifen has not been clarified. It may have a direct anti-estrogenic effect in gastric tissue. Women who receive tamoxifen may be those with high ERα in their breasts and they may therefore also have a higher level of ERα in their gastric mucosa, and thus be more vulnerable to blockage of ERs. Another suggested mechanism is through inhibition of the binding of histamine to cytochrome P450.
enzymes. A histamine-P450 interaction could disturb normal homeostatic maintenance of intracellular levels of lipid mediators. These mediators modulate gene function, including expression of the cytochrome P450, and thus affect cell growth and proliferation. Moreover, tamoxifen has been found to regulate expression of transforming growth factor-α and β, and to bind to calcium channels and protein kinase C, but it is not known through which of these mechanisms, if any, it acts on the gastric mucosa.

Although not all researchers agree that sex hormone receptors are involved in gastric carcinogenesis, and our studies did not produce any strong evidence of a role for ERs in explaining the estrogen relation to gastric AC development, this possibility should not be rejected. The decrease in the expression of ERβ in gastric AC that we observed is of particular interest, since this receptor has been linked to promotion of epithelial differentiation and plays a role in the organization and architectural maintenance of the colon. It may have a similar function in the gastric mucosa. The significance of the presence of ERβcx in gastric adenocarcinoma remains to be further investigated. ERβcx does not bind oestrogen; but rather it inhibits ERα from binding DNA, while it does not influence ERβ. It is hoped that future research that can establish the potential role of estrogen and ERs in relation to gastric adenocarcinoma might open the way for adjuvant or preventive strategies in the treatment of this cancer, for example through the action of more selective ER modulators, and probably of specific ERβ agonists.
Estrogen in the Development of Esophageal and Gastric Adenocarcinoma
Conclusions

Hormone replacement therapy with estrogen might reduce the risk of gastric adenocarcinoma by as much as 50%. This effect seems stronger for non-cardia gastric cancer.

We provided no evidence in favor of an association between hormone replacement therapy with estrogen and risk of esophageal adenocarcinoma.

Use of the anti-estrogen tamoxifen might increase the risk of non-cardia gastric adenocarcinoma.

Tamoxifen use might accelerate the development of gastric adenocarcinoma in patients with previous breast cancer.

Tamoxifen use does not seem to increase the risk of esophageal adenocarcinoma.

The intestinal type of gastric adenocarcinoma seems to be less frequent in women with high endogenous estrogen exposure compared to men and women without such exposure.

There seem to be no substantial differences in the expression of estrogen receptor alpha, beta and beta cx between groups of patients categorized with regard to endogenous estrogen exposure.

Tamoxifen use does not seem to have any material influence on the distribution of the two histological types of gastric adenocarcinoma.

The expression of estrogen receptor beta cx has been identified for the first time in gastric tissue.

The expression of estrogen receptor alpha seems to be increased in gastric adenocarcinoma, while the expression of estrogen receptor beta and beta cx seems to be decreased, compared to that in adjacent non-tumor tissue.
Future research

Some of the findings described in this thesis will provide reason for further research. The presence of estrogen receptors in various groups of patients with esophageal AC has not been investigated, but might be of interest. No data are available concerning the occurrence of estrogen receptors in the esophagus and stomach in the normal population. We did studied these receptors in the non-cancerous tissue adjacent to gastric AC, but future research should focus on mapping out ERs in the esophagus and stomach of healthy individuals. Currently, such research by our group is in the planning stages. If further evidence supports the hypothesis that estrogen protects against gastric AC, then randomized, controlled trials should be considered. Patients with gastric AC with a particular composition of ERs could be randomized to adjuvant treatment with estrogen or with a selective estrogen receptor modulator.
Popular-scientific summary in Swedish
Populärvetenskaplig sammanfattning på svenska

Bakgrund
Cancer består av mer än 100 olika sjukdomar och alla vävnader i kroppen kan angrippas. Enligt Världshälsoorganisationen (WHO) dog mer än 7.5 miljoner människor av cancer år 2005 och denna siffra beräknas stiga till 9 miljoner år 2015.


I denna avhandling har vi i olika studier testat hypotesen att östrogen skyddar kvinnor mot esofagus- och ventrikeladenocarcinom.

Studie I
Vi använde oss av en stor databas i Storbritannien, General Practice Research Database, som har patientdata från ett stort antal vårdcentraler. En s. k. fall-kontrollstudie utfördes. Alla kvinnor som efter klimakteriet drabbades av esofagus- eller ventrikeladenocarcinom mellan 1994-2001 identifierades och jämfördes med avseende på hormonsubstitution med östrogen (HRT) med en kontrollgrupp av kvinnor utan dessa diagnoser. Slutsatsen blev att kvinnor med HRT löper en ca 50% mindre risk att drabbas av ventrikeladenocarcinom. Riskminskningen var ännu mer uttalad (66%) för ventrikeladenocarcinom nedom övre magmunnen (non-cardia). Risken för esofaguscancer var inte påverkad.

Studie II
Signifikant större (27%, avser risken för cancer i non-cardia) och denna risk ökade med tiden efter bröstcancerdianosen. Risken för esofagusadeno- carcinoform var inte statistiskt signifikant ökad hos tamoxifenexponerade.

**Studie III**


**Studie IV**

I den sista studien identifierades kvinnor som först hade drabbats av bröstcancer och som senare i livet drabbades även av ventrikeladenocarcinom. Vid journalgenomgång så kännetecknades diagnosen och eventuell tamoxifenbehandling mot bröstcancer. Vi analyserade tumörmaterial och hittade ingen stor skillnad mellan de två patientgrupperna vad det gäller histologin (intestinal/diffus ventrikeladenocarcinom). Östrogenreceptornas sammansättning i den intestinala typen var också lika mellan grupperna. Däremot var tiden mellan ventrikel- och bröstcancerdiagnoserna för patienter med tamoxifen behandling kortare jämfört med dem utan sådan behandling.

**Slutsatser**

I denna avhandling har vi kunnat konstatera att:

- Hormonsubstitution med östrogen verkar kunna minska risken för ventrikeladenocarcinom, särskilt för tumörer nedanför övre magmunnen av ventrikeln.
- Tamoxifenbehandling verkar kunna öka risken för ventrikeladenocarcinom.
- Tamoxifen verkar inte påverka fördelningen av den histologiska typen av ventrikeladenocarcinom (intestinal/diffus).
- Tamoxifen verkar möjligen kunna påskynda utveckling av ventrikelcancer.
- Den intestinala typen av ventrikeladenocarcinom verkar vara mindre vanlig hos östrogenexponerade kvinnor jämfört med oxponerade män och kvinnor.
- Östrogenreceptor beta bcx kan finnas i magsäcken.
- Vi hittade inga större skillnader i östrogenreceptorsammansättning mellan olika patientgrupper med avseende på deras östrogenexponering.
- I ventrikeladenocarcinom uttrycks östrogenreceptor alfa i större utsträckning medan beta och beta cx i mindre, jämfört med närliggande vävnad.
Περίληψη στα Ελληνικά

Εισαγωγή
Ο καρκίνος είναι μια από τις αρχαιότερες ασθένειες της ανθρωπότητας. Ο Ιπποκράτης (460-370 π.Χ.) περιγράφει τον χαρακτηριστικό τρόπο ανάπτυξης των όγκων που μοιάζει με τις διαγκάνες κάβουρα και βασιζεί την ασθένεια με το όνομα καρκίνος, λέξη που στην αρχαία ελληνική σημαίνει κάβουρα.

Ο καρκίνος σήμερα συντίθεται από περισσότερες από 100 διαφορετικές ασθένειες και μπορεί να προσβάλει οποιονδήποτε ιστό του σώματος. Σύμφωνα με τον Διεθνή Οργάνωση Υγείας, το 2005 σημειώθηκαν περισσότεροι από 7,5 εκατομμύρια θάνατοι εξαιτίας του καρκίνου. Η πρόβλεψη για το 2015 ανέρχεται σε 9 εκατομμύρια θανάτους.

Το αδενοκαρκίνομα του οισοφάγου είναι ο καρκίνος με την μεγαλύτερη αύξηση στον Δυτικό κόσμο, ενώ το αδενοκαρκίνομα του στομάχου είναι η πιο συνήθιστη μορφή στομαχικού καρκίνου. Χαρακτηριστικό και των δύο αυτών μορφών καρκίνου είναι ότι εμφανίζονται συχνότερα στους άνδρες απ’ ότι στις γυναίκες. Σε ορισμένες περιπτώσεις του κόσμου, ανά μια γυναίκα με αδενοκαρκίνομα στον οισοφάγο αντιστοιχούν έως και 8 άνδρες με την ίδια νόσο, ενώ τουλάχιστον 2 είναι οι άνδρες που αντιστοιχούν σε κάθε γυναίκα με αδενοκαρκίνομα στο στομάχι. Η αναγνώριση αυτής ανάλυσης μεταξύ των δύο φύλων παραμένει ανεξήγητη.

Τα οιστρογόνα συνιστούν κατά κύριο λόγο γυναικείες ορμόνες που παράγονται στις οσοθήκες, με τα επίπεδά τους στο σάμα να αυξάνονται κατά την έναρξη της εφηβείας και να ελαττώνονται κατά την εμμηνόπαυση. Πειράματα σε ζώα αλλά και επιδημειολογικές μελέτες, τείνουν στο συμπέρασμα ότι, ο χαμηλός αριθμός περιστατικών αδενοκαρκινώματος στο οισοφάγο και στομάχι στις γυναίκες, ίσως οφείλεται σε ορμονικούς παράγοντες.

Στην παρούσα Διδακτορική Διατριβή που αποτελείται από τέσσερις επιστημονικές μελέτες, διερευνάται η υπόθεση ότι τα οιστρογόνα προστατεύουν τις γυναίκες από αυτές τις δύο μορφές καρκίνου. Για τον σκοπό αυτόν τη χρησιμοποιήθηκαν τόσο επιδημιολογικές μελέτες, όσο και εργαστηριακή έρευνα.

Μελέτη 1
Στην πρώτη μας μελέτη χρησιμοποιήσαμε μια εκτεταμένη βάση δεδομένων από τη Μεγάλη Βρετανία (General Practice Research Database), στην οποία καταχωρούνται πληροφορίες για ασθένειες από έναν μεγάλο αριθμό Κέντρων Υγείας. Από το 1994 έως το 2001 πραγματοποιήσαμε συγκρίσεις μεταξύ γυναικών στην κλιμακτήριο που προσβλήθηκαν από αδενοκαρκινόμα στον οισοφάγο ή στο στομάχι με γυναίκες, αντιστοιχής ηλικίας, από την ίδια βάση δεδομένων, χωρίς αυτές τις μορφές καρκίνου. Διαπιστώσαμε ότι η ορμονική θεραπεία με οιστρογόνα μειώνει κατά 52% τον κίνδυνο προσβολής από αδενοκαρκινόμα στο στομάχι ενώ, ελαττώνεται κατά 66%, ο κίνδυνος προσβολής από αδενοκαρκίνομα εκτός της καρδιάς στομάχου (δηλαδή όχι κοντά στην ένωση του στομάχου με τον οισοφάγο). Ο κίνδυνος προσβολής από οισοφαγικό καρκίνο δεν φαινόταν στην κατμαρμένη σε κανένα από τα ορμονικά.
νεται να επηρεάζεται από την ορμονική θεραπεία.

Μελέτη 2ον
Εάν τα οιστρογόνα προστατεύουν τις γυναίκες από το αδενοκαρκίνωμα του οισοφάγου και του στομάχου, τότε τα αντιοιστρογόνα θα πρέπει να ανεξάρτητα τον κίνδυνο προσβολής από τους προανεφερθείσες καρκίνους. Στην 2ον εργασία μας, εξετάσαμε τον κίνδυνο προσβολής σε γυναίκες που είχαν υποβληθεί σε θεραπευτική αγωγή με το αντιοιστρογόνο παράσκευσημα της Συνήθης θεραπευτικής αγωγής στην Σουηδία. Για την μελέτη αυτή χρησιμοποιήσαμε δεδομένα από το Σουηδικό Μητρώο Καρκίνου. Όλες οι γυναίκες με διάγνωση καρκίνου του μαστού από το 1961 έως το 2003 αναγνωρίσθηκαν. Εκλάβαμε ως δεδομένο ότι οι ασθενείς με διάγνωση που πραγματοποιήθηκε το έτος 1988 ή μεταγενέστερα είχαν υποβληθεί σε θεραπευτική αγωγή με της Συνήθης ασθενείς με καρκίνο μαστού που διαγνώσθηκε όπως υποβληθεί σε τέτοια θεραπεία. Συγκρίναμε έκαστη εξ αυτών των χρονικά οριοθετημένων ημερών με τον υπόλοιπο γενικό πληθυσμό, υπολογισμό αναφορικά με τον κίνδυνο προσβολής από αδενοκαρκίνωμα οισοφάγου και στομάχου. Σε σύνολο περισσότερων από 138 000 ασθενών με καρκίνο του μαστού διαπιστώσαμε ότι η ταμοξιφένη αυξάνει τον κίνδυνο αδενοκαρκίνωμα στομάχου κατά 27% (στην περιοχή εκτός καρδιάς), ενώ ο κίνδυνος αδενοκαρκίνωμα στον οισοφάγο δεν φαίνεται να επηρεάζεται.
νονται στον καρκινικό ιστό, εν συγκρίσει με τον μη καρκινικό ιστό που γειτνιάζει με αυτόν.

Μελέτη 49
Στην τελευταία μας εργασία εξετάσαμε περιπτώσεις γυναικών στη Στοκχόλμη, μεταξύ των ετών 1958-2005, στις οποίες διαγνώστηκε πρώτα, καρκίνος του μαστού και κατόπιν (μετά την πάροδο τουλάχιστον ενός έτους) διαγνώστηκε αδένοκαρκίνωμα του στομάχου. Οι ασθενείς ήταν καταχωρημένοι στο Σουηδικό Μητρώο Καρκίνου. Συλλέξαμε τα ιστορικά των ασθενών και επιβεβαιώσαμε τις διαγνώσεις καθώς και ενδεχόμενη θεραπευτική σχεδιάση με ταμοξιφένη. Συνολικά συμπεριλάβαμε 68 ασθενείς και συγκεντρώσαμε ιστολογικό υλικό για την μελέτη υποδοχέων οιστρογόνου. Οι ασθενείς που είχαν υποβληθεί σε θεραπεία με ταμοξιφένη δεν παρουσίαζαν διαφορά από τις ασθενείς που δεν είχαν υποβληθεί σε τέτοια θεραπεία, στην αναλογία αδένοκαρκινώματος τύπου εντερικού/διάχυτου και την ύπαρξη υποδοχέων οιστρογόνου (στον εντερικό τύπο). Ωστόσο οι ασθενείς που είχαν υποβληθεί σε θεραπεία με ταμοξιφένη προσβλήθηκαν από αδένοκαρκινώμα στο στομάχι μέσα σε πολύ λιγότερο χρόνο, από όσον δεν είχαν λάβει ταμοξιφένη (4 έτη αντί 10).

Συμπεράσματα
Τα συμπεράσματα της παρούσας Διδακτορικής Διατριβής έχουν συνοπτικά ως εξής:
• Η ορμονική θεραπεία με οιστρογόνα σε γυναίκες στην εμμηνόπαυση φαίνεται ότι μειώνει τον κίνδυνο εκδήλωσης αδένοκαρκινώματος στο στομάχι και ιδιαίτερα εκτός της περιοχής της καρδίας στομάχου.
• Η θεραπεία με ταμοξιφένη φαίνεται πως αυξάνει τον κίνδυνο εμφάνισης αδένοκαρκινώματος στο στομάχι.
• Η ταμοξιφένη δεν επηρεάζει την αναλογία εντερικού/διάχυτου τύπου αδένοκαρκινώματος του στομάχου.
• Η ταμοξιφένη φαίνεται να επιταχύνει την εκδήλωση καρκίνου στο στομάχι.
• Ο εντερικός τύπος αδένοκαρκινώματος στο στομάχι παρουσιάζεται σπανιότερα σε γυναίκες που είναι εκτεθειμένες σε οιστρογόνα, συγκριτικά με άνδρες και γυναίκες μη εκτεθειμένους σε αυτά.
• Ο υποδοχέας οιστρογόνου βεχ υπάρχει στο στομάχι.
• Δεν βρέθηκαν ουσιαστικές διαφορές μεταξύ των τριών ομάδων ασθενών σε ότι αφορά την ύπαρξη οιστρογονικών υποδοχέων α, β, και βεχ.
• Οι υποδοχέες α αυξάνονται, ενώ οι β και βεχ, μειώνονται στον καρκινικό ιστό (αδένοκαρκίνωμα στομάχου) σε σύγκριση με τον γειτνιάζοντα μη καρκινικό ιστό.
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We do not so much need the help of our friends as the confidence of their help in need.
Epicurus

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