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**DIETARY  
PHYTOESTROGENS AND  
ESOPHAGEAL CANCER**

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## ABSTRACT

Esophageal cancer is the eighth most common invasive cancer in the world, a cancer with an increasing incidence and male predominance, and there is a great need for potential dietary prevention. The overall aim of this thesis was to evaluate whether the dietary phytoestrogens lignans might play a protective role in the etiology of esophageal cancer, including gastroesophageal junctional adenocarcinoma.

In **Paper I**, we examined the association between intake of dietary lignans based on a 63-item food frequency questionnaire (FFQ) and risk of esophageal cancer in a Swedish nationwide population-based case-control study conducted in 1995-1997. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Participants in the highest quartile of lignan intake compared with the lowest quartile showed a decreased risk of esophageal adenocarcinoma (OR=0.65; 95% CI: 0.38-1.12) and gastroesophageal junctional adenocarcinoma (OR=0.37; 95% CI: 0.23-0.58), while no clear associations were found for esophageal squamous-cell carcinoma.

In **Paper II**, we validated the use of two FFQs (the 67-item FFQ-87 and the 93-item FFQ-97) for the assessment of dietary lignans compared to the serum biomarker enterolactone, the main metabolite of dietary lignans in the human body. Based on the FFQ-97, the correlation between lignan intake and serum enterolactone was significant, but the value of the correlation coefficient was small ( $r=0.22$ ,  $p=0.01$ ). No significant correlation was observed for the FFQ-87.

In **Paper III**, we further evaluated the possible association between lignan intake based on the FFQ-97 and risk of esophageal and gastric adenocarcinoma using a prospective study design. Among 81,670 participants who were followed up during 1998-2009, hazard ratios (HRs) and 95% CIs were calculated. No statistically significantly decreased risk was found. Compared with the lowest quartile of lignan intake, the adjusted HRs of the highest quartile were 0.96 (95% CI: 0.46-2.00) for esophageal and gastroesophageal junctional adenocarcinoma, and 0.89 (95% CI: 0.52-1.55) for gastric adenocarcinoma.

In **Paper IV**, we defined a dietary pattern characterized by dietary intake of lignans, quercetin and resveratrol, the three common phytochemicals with estrogenic properties, in a Swedish population-based case-control study. A decreased risk of esophageal cancer was found among individuals with a high dietary intake of these three phytochemicals. Comparing the highest quintile of food pattern score with the lowest quintile, the adjusted ORs were 0.24 (95% CI: 0.12-0.49) for esophageal adenocarcinoma, 0.31 (95% CI: 0.15-0.65) for esophageal squamous-cell carcinoma, and 0.49 (95% CI: 0.28-0.84) for gastroesophageal junctional adenocarcinoma.

In conclusion, a high dietary intake of phytoestrogens, typically lignans, might decrease the risk of adenocarcinoma of esophagus and gastroesophageal junction. The FFQ-97 can be used to assess lignan exposure, and a dietary pattern characterized by a high dietary intake of lignans, quercetin, and resveratrol might prevent esophageal cancer.

# LIST OF PUBLICATIONS

- I. Lin Y, Yngve A, Lagergren J, Lu Y.  
**Dietary intake of lignans and risk of adenocarcinoma of the esophagus and gastroesophageal junction.**  
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- II. Lin Y, Wolk A, Håkansson N, Peñalvo JL, Lagergren J, Adlercreutz H, Lu Y.  
**Validation of FFQ-based assessment of dietary lignans compared with serum enterolactone in Swedish women.**  
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- III. Lin Y, Wolk A, Hakansson N, Lagergren J, Lu Y.  
**Dietary intake of lignans and risk of esophageal and gastric adenocarcinoma: a cohort study in Sweden.**  
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- IV. Lin Y, Yngve A, Lagergren J, Lu Y.  
**A dietary pattern rich in lignans, quercetin and resveratrol decreases the risk of esophageal cancer.**  
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## LIST OF ABBREVIATIONS

HRT	Hormone replacement therapy
ER	Estrogen receptor
CVD	Cardiovascular disease
PIN	Pinoresinol
LAR	Lariciresinol
SEC	Secoisolariciresinol
MAT	Mataireinol
SYR	Syringaresinol
MED	Medioresinol
TR-FIA	Time-resolved fluoroimmuno assay
ENL	Enterolactone
END	Enterodiol
BMI	Body mass index
RRs	Relative risks
ORs	Odds ratios
CI	Confidence intervals
SECC	Swedish Esophageal and Cardia Cancer Study
SMC	Swedish Mammography Cohort
COSM	Cohort of Swedish Men
SHBG	Sex hormone-binding globulin

# 1 INTRODUCTION

Esophageal cancer is one of the least studied and deadliest cancers worldwide. There are two main histologic types of esophageal cancer: squamous-cell carcinoma and adenocarcinoma. Esophageal adenocarcinoma is the dominant histological subtype of esophageal cancer in Western society. The abruptly increasing incidence and the strong male predominance (3 to 9:1) of esophageal adenocarcinoma have attracted considerable attention during the last few decades.<sup>1,2</sup> The prognosis of esophageal cancer is poor, although surveillance, diagnostic measures and therapy have improved. Given the rapidly rising incidence and poor survival, it is important to identify potentially modifiable beneficial factors for esophageal cancer, particularly for men.

Dietary phytoestrogens are a group of compounds that naturally occur in plant foods with estrogenic properties due to similar chemical structures to endogenous estrogens. They have also been observed to have other beneficial effects, such as anti-oxidative, anti-carcinogenic and anti-obesity properties.<sup>3,4</sup> A number of nutritional epidemiological studies have demonstrated the protective role of dietary phytoestrogen in several cancers, including breast, prostate, colon and ovarian cancer.<sup>5-9</sup> Evidence from *in vitro* as well as *in vivo* studies has suggested a potential anti-carcinogenic effect of phytoestrogen in esophageal tumor cell lines.<sup>10</sup> However, no epidemiological study has, to the best of my knowledge, investigated the potential association between phytoestrogen lignan intake and risk of esophageal cancer.

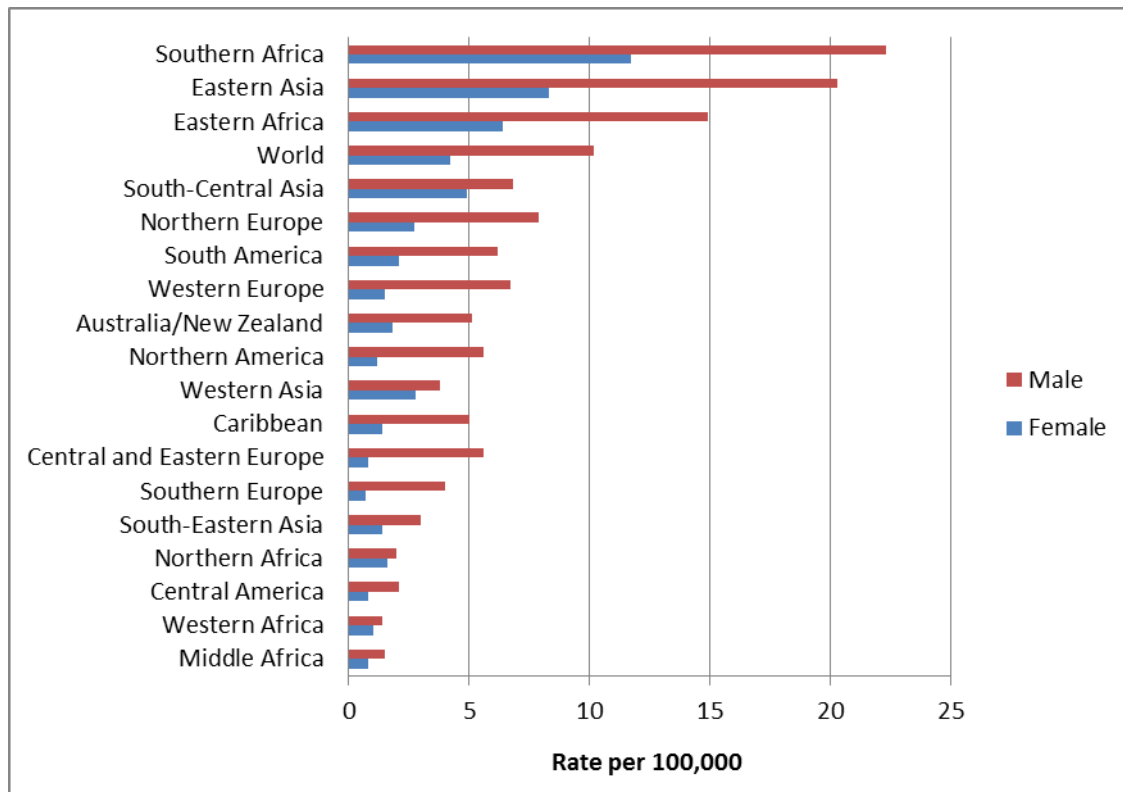
Given this deficit of epidemiological information about a possible phytoestrogen-esophageal cancer relation, the aim of this thesis was to clarify the associations of phytoestrogen intake with risk of human esophageal cancer, including gastroesophageal junctional adenocarcinoma.

## 2 BACKGROUND

### 2.1 ESOPHAGEAL CANCER

#### 2.1.1 Incidence and trend

Worldwide, esophageal cancer is the eighth most frequent cancer, with an estimated 481,000 new cases (3.8% of the total) diagnosed in 2008, and the sixth leading cause of death from cancer, with 406,000 deaths (5.4% of the total).<sup>11</sup> However, esophageal cancer is mainly a disease of low-income countries, occurring around four times more frequently in low- to middle- than in high-income countries. The highest risk areas of the world are in southern Africa, with an age-standardized incidence of up to 22.3 per 100,000 in men and 11.7 per 100,000 in women (**Figure 1**).<sup>11</sup> High incidences are also reported in the Asian ‘esophageal cancer belt’, stretching from northern Iran through the central Asian republic to north-central China.

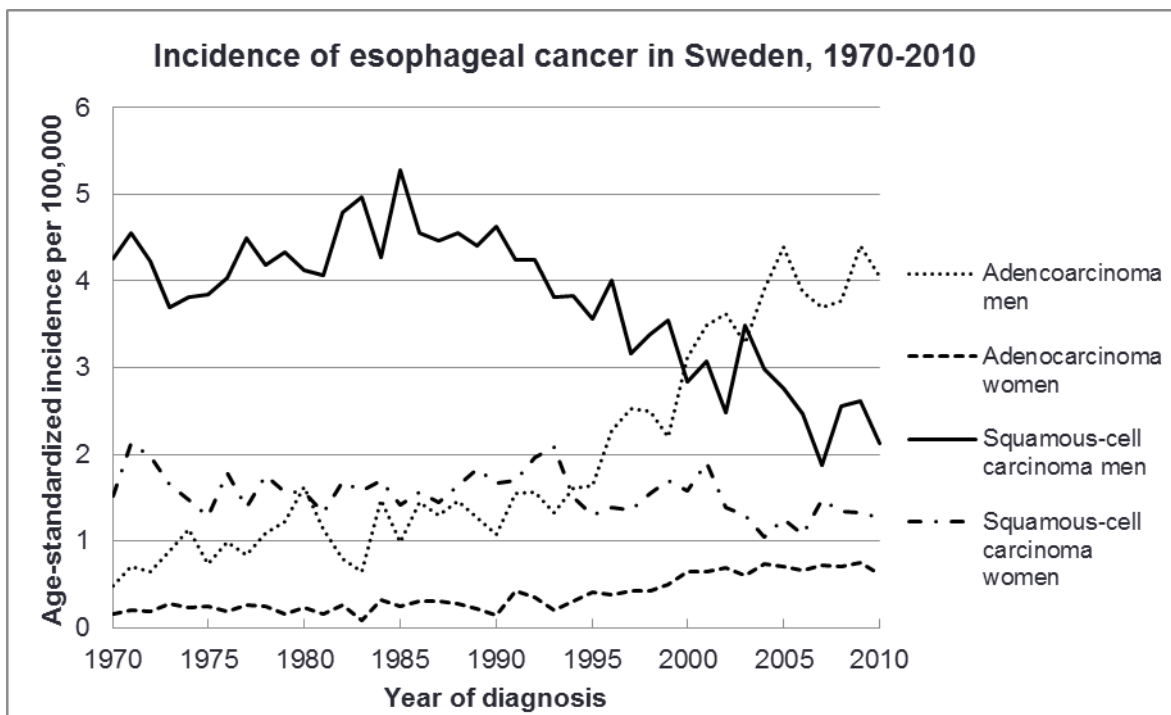


**Figure 1.** Esophageal Cancer, World Age-Standardized Incidence Rates, World Regions, 2008 Estimates.<sup>11</sup>

There are two main histological types of esophageal cancer: squamous cell carcinoma and adenocarcinoma. Squamous cell cancer arises from the epithelial cells that line the upper part of the esophagus, and adenocarcinoma arises from glandular cells that are present at the junction of the esophagus and stomach.<sup>12</sup> From a global perspective, squamous cell carcinoma is the dominant subtype of esophageal cancer, particularly in



Asian countries, accounting for over 90% of all esophageal cancer cases.<sup>13</sup> High incidence areas of esophageal squamous-cell carcinoma include Normandy and Bretagne in Europe, and northern China, Japan, India and Iran.<sup>14,15</sup> The incidence rates of squamous-cell carcinoma have remained stable or decreased in Western countries in recent decades.<sup>1</sup> Specifically, the overall age-standardized incidence of squamous-cell carcinoma in Sweden has dropped from 4.26 per 100,000 in 1973 to 2.13 in 2010 in men, and in women from 1.52 to 1.26 per 100,000 (**Figure 2**).<sup>16</sup> Meanwhile, in the latter half of the 1980s, it was realized that the incidence of esophageal adenocarcinoma was increasing rapidly, especially for men, in many Western populations.<sup>17-27</sup> In Sweden, the age-standardized incidence of esophageal adenocarcinoma increased from 0.48 per 100,000 in 1973 to 4.05 in 2010 in men, and in women from 0.16 to 0.62 per 100,000 (**Figure 2**).<sup>16</sup> The rapidly increasing incidence of esophageal adenocarcinoma is a global phenomenon.<sup>2</sup> A recent global assessment of 117,946 incident cases of esophageal adenocarcinoma found that the average annual increase ranged from 3.5% in Scotland to 8.1% in Hawaii.<sup>28</sup>



**Figure 2.** Trends in age-standardized incidence of esophageal cancer per 100,000 during 1970 and 2010 in Sweden.<sup>16</sup>

The gastroesophageal junction forms the border between the distal esophagus and the proximal stomach, and normally is where the squamous epithelium of the esophagus transitions into the columnar epithelium of the gastric cardia (the Z-line).<sup>29</sup> To be classified as carcinoma of the gastroesophageal junction, the tumor has to have its center within 5 cm proximal and distal of the anatomic gastric cardia.<sup>30</sup> The majority of neoplasms diagnosed at the gastroesophageal junction are adenocarcinoma. Gastroesophageal junctional adenocarcinoma shows similarities with esophageal adenocarcinoma in etiology and the anatomical proximity, and therefore they are frequently considered together. Gastroesophageal junctional adenocarcinoma is a rare

but lethal condition with increasing incidence since the early 1970s. The age-adjusted incidence rate consistently increased from 1.22 per 100,000 person years during 1973-1978 to 1.94 during 2003-2008 in the USA.<sup>31</sup>

## 2.1.2 Survival

Although surveillance, diagnostic measures and therapy have improved, the overall 5-year survival in patients with esophageal cancer remains lower than 15% in Western societies.<sup>1</sup> Lagergren *et al.* reported that relative 5-year survival rates during the two recent periods 1990-1999 and 2000-2008 were 12.5% and 10.3% for esophageal squamous-cell carcinoma, and 12.5% and 14.6% for esophageal adenocarcinoma in the Swedish population.<sup>32</sup> Recent data collected from nine Surveillance, Epidemiology, and End Results Registries in USA indicate moderate improvements in survival of total esophageal cancer, as well as gastroesophageal junctional adenocarcinoma.<sup>33,34</sup> The cumulative survival of gastroesophageal junctional adenocarcinoma increased from 8% to 12% to 17% diagnosed during 1973-1984, 1985-1996, and 1997-2008, respectively.<sup>31</sup> The data from Sweden also illustrates the similar survival increase of gastroesophageal junctional adenocarcinoma from 11.1% to 14.2% during the period 1990-1999 to 2000-2008.<sup>32</sup> These changes might be related to improvements in endoscopic detection and in surgical and medical therapy for early-stage disease.<sup>35</sup>

## 2.1.3 Risk factors in brief

A summary of risk factors for both histological types of esophageal cancer, and gastroesophageal junctional adenocarcinoma are presented in **Table 1**.<sup>36,37</sup>

### 2.1.3.1 Adenocarcinoma

#### **Gastroesophageal reflux disease and Barrett's esophagus**

Gastroesophageal reflux disease is an established strong and dose-dependent risk factor for esophageal adenocarcinoma and it affects up to 30% of the Western population on a monthly basis.<sup>38</sup> Longstanding reflux is the main cause of Barrett's esophagus, which is a precursor to esophageal adenocarcinoma, and the strongest known risk factor for this malignancy. Barrett's esophagus is characterized by the replacement of the normal squamous epithelium in the distal esophagus with an intestinal-like specialized columnar epithelium.<sup>39,40</sup> A recent study found that the relative risk of esophageal adenocarcinoma among patients with Barrett's esophagus was 11.3, compared with the risk in the general population.<sup>41</sup>

#### **Obesity**

Obesity is established as an important risk factor of esophageal and gastroesophageal junctional adenocarcinoma and it might be the key factor explaining the increasing incidence and male predominance of these tumors.<sup>42,43</sup> Lagergren *et al.* found that the heaviest quartile of the population had a 7.6-fold increased risk of esophageal

adenocarcinoma.<sup>44</sup> It should be noted that not only body mass index (BMI), but more importantly, visceral obesity is strongly associated with increased risk. Visceral obesity, also named as abdominal obesity or central obesity, is the accumulation of abdominal fat resulting in an increasing waist circumference. Recent studies indicate that visceral obesity increases the occurrence of reflux and Barrett's esophagus independently of BMI.<sup>45</sup> Furthermore, visceral obesity is more common in men than in women, which might contribute to the male predominance of esophageal adenocarcinoma. A potential underlying mechanism is that visceral obesity increases intra-abdominal pressure, which leads to increased intra-gastric pressure. This leads to an increased gastroesophageal pressure gradient inducing lower esophageal sphincter relaxation, and finally causes gastroesophageal reflux.<sup>46,47</sup>

### **Tobacco smoking**

Tobacco smoking is a moderate risk factor for esophageal adenocarcinoma and could increase the risk at a level ranging from 1.5-fold to 4-fold.<sup>35,37,48</sup> A recent meta-analysis reported that the pooled risks are approximately 2-fold higher in ever-smokers compared to never-smokers.<sup>49</sup> Smoking patterns (cigarette consumption and duration of smoking) also influenced the cancer risk differently. Compared to never-smokers, those who smoked  $\geq 20$  cigarettes/day or had a long smoking duration ( $\geq 40$  years) had a 2.5-fold increased risk.<sup>49</sup>

### **Alcohol intake**

The risk of esophageal adenocarcinoma is not or only weakly related to alcohol consumption. Although some early studies have found that alcohol consumption is associated with an increased risk of esophageal adenocarcinoma,<sup>50,51</sup> a recent meta-analysis provides evidence of an absence of any such association.<sup>52</sup> In an earlier study based on the same database as **Paper I** and **IV**, Lagergren *et al.* concluded that the risk of esophageal adenocarcinoma was not associated with alcohol use.<sup>53</sup> Interestingly, it is often believed that light or moderate alcohol consumption is associated with a reduced risk of esophageal cancer. Those who drank modest levels of wine (<50-90/week) or port or spirits (<10-20 g/week) had significantly lower risks of esophageal adenocarcinoma in a nationwide Australian case-control study.<sup>54</sup>

### **Fruit and vegetables**

Several studies have suggested that high consumption of fruit and vegetables could be considered as protective factors against esophageal cancer.<sup>39,55-59</sup> Low intake of fruit and vegetables has been estimated to account for 15.3% of esophageal adenocarcinoma.<sup>60</sup> Interestingly, the potential beneficial effects of vegetables and fruit may be specific to certain groups, including dark green, leafy green or raw vegetables, raw fruits and citrus fruits.<sup>55,61-64</sup>

### **Fiber intake**

Dietary fiber intake has been widely suggested as a strong and independent beneficial factor in the development of esophageal adenocarcinoma.<sup>58,62,65-68</sup> It may have

protective effects via a mechanical action by helping to remove and/or limit the contact of carcinogens with the esophageal epithelium.<sup>69</sup> In addition, high-fiber foods generally tend to have a higher content of antioxidants and phytochemicals.<sup>70,71</sup> It has also been hypothesized that high-fiber intake may reduce the risk of hiatal hernia, erosive esophagitis and reflux symptoms, which have been implicated as risk factors for esophageal adenocarcinoma.<sup>68,72</sup> Particularly cereal fiber, representing a major source of fiber intake (>60%) in Swedish population, plays a major protective role in the etiology of esophageal adenocarcinoma.<sup>73</sup>

### **Fat and meat**

Several studies based on Western populations reported an increasing risk of esophageal adenocarcinoma following high consumption of total meat or red meat. However, these results were not statistically significant.<sup>63,64,66,68,74</sup> In terms of processed meat, Gonzales *et al.* reported a 3-fold increased risk of adenocarcinoma among those with high consumption, using the data from the EPIC cohort.<sup>74</sup> One of the largest case-control studies in UK suggested that total fat, saturated fat, and mono-unsaturated fat intakes were adversely associated with risk of esophageal adenocarcinoma.<sup>75</sup> It has been hypothesized that high dietary fat intake might exert harmful effects by inducing a significant increase in taurine-conjugated bile acids in the bile juice, and by increasing the pH in the esophagus, as well as by increasing the frequency of transient lower esophageal sphincter relaxations.<sup>76-78</sup> El-Serag *et al.* have supported these hypotheses by reporting that high dietary fat intake was associated with an increased risk of erosive esophagitis and reflux symptoms in a cross-sectional study.<sup>72</sup>

### **Phytoestrogens**

Studies on phytoestrogen intake and esophageal cancer are sparse. A large scale case-control study in USA observed a 57% decreased risk of esophageal squamous-cell carcinoma with greater isoflavone consumption.<sup>79</sup> No association between isoflavone intake and esophageal adenocarcinoma was detected, though. However, another study in USA indicated a reduced risk of Barrett's esophagus, a precursor of esophageal adenocarcinoma, comparing the highest tertile of isoflavone intake with the lowest category.<sup>80</sup>

### **Sex hormones and reproductive factors**

The female sex hormones, mainly estrogens, have been postulated to play a protective role in the etiology of esophageal cancer.<sup>81</sup> Estrogen might exert protective effects via estrogen receptors, which have been observed in esophageal tissue. However, only a few epidemiologic studies have addressed this hypothesis and most of the available studies have failed to reveal any such associations. In population-based studies, no influence of hormone replacement therapy,<sup>82</sup> other estrogen therapy,<sup>83</sup> or childbearing<sup>84</sup> have been reported. However, breastfeeding has been found to be a protective factor,<sup>57</sup> while high parity and low age at first delivery were also indicated to be beneficial from this respect.<sup>85,86</sup> In a recent pooled analysis combining data from several large case-control studies, a reduced risk of esophageal and gastric junctional adenocarcinoma was confirmed among women who breastfed and the risk decreased

with increased duration of breastfeeding.<sup>83</sup> The potential influence of other endogenous reproductive factors, such as menstruation, history of pregnancy, and exogenous factors, including use of hormone replacement therapy and of oral contraceptives however were not found in that study.<sup>83</sup>

### **Other risk factors**

High socioeconomic status, male gender,<sup>87</sup> Caucasian race<sup>88</sup> have also been reported to increase the risk of esophageal adenocarcinoma. *H. pylori* infection has shown to have a protective role against esophageal adenocarcinoma.<sup>89</sup>

#### *2.1.3.2 Squamous-cell carcinoma*

Numerous epidemiologic studies have established that alcohol consumption and tobacco smoking are causal risk factors for esophageal squamous-cell carcinoma.<sup>90,91</sup> Alcohol, by virtue of its oral intake, will reach every cell of the body; nevertheless, some of the effects on the gastrointestinal tract are direct. After consumption, alcohol is metabolized by oxidation to acetaldehyde, which has direct carcinogenic and mutagenic effects by modifying DNA via generation of DNA adducts.<sup>92</sup> In Western countries, about 90% of esophageal squamous-cell carcinomas are considered to be caused by a combination of alcohol consumption and tobacco smoking.<sup>93</sup> Among heavy drinkers ( $\geq 12$  drinks per week), relative risks (RRs) range from 2.9 to 7.4,<sup>48,94</sup> while a recent meta-analysis showed that moderate consumption ( $\leq 7$  drinks per week) was associated with 30% increased risk of esophageal squamous-cell carcinoma.<sup>95</sup> In addition to alcohol, smoking greatly increases the risk of squamous-cell carcinoma. Prospective epidemiologic data observed a 5-fold higher risk among smokers compared to nonsmokers, with risk for heavy smokers increasing nearly 10-fold.<sup>48</sup> Furthermore, many case-control studies have shown synergistic effects of alcohol and tobacco smoking in the development of esophageal squamous-cell carcinoma.<sup>37,96</sup>

Early evidence from case-control studies show moderate (20-60%) reductions in risk with high compared with low fruit and vegetable consumption.<sup>56,97</sup> Recently, a meta-analysis study reported that the highest intake of both vegetables and fruit can decrease the risk by 50% in contrast with lowest intake. They also observed a non-linear association for intake of both fruit ( $P_{\text{non-linearity}} < 0.001$ ) and vegetables ( $P_{\text{non-linearity}} = 0.04$ ).<sup>98</sup> Together with the evidence present in reviews and meta-analysis, there is very strong consistency in the evidence for a protective role of fruit and vegetables in squamous-cell carcinoma. Of note, pickled vegetables, widely consumed in China, have been suggested as a potential risk factor for high incidence of squamous-cell carcinoma in this area.<sup>99,100</sup> The effect of tea and coffee intake on squamous-cell carcinoma is unclear.<sup>101</sup> However, study from East-Asian countries, especially China,

**Table 1.** Summary of current knowledge of risk factors of the development of esophageal cancer.<sup>36,37</sup>

	<b>Esophageal adenocarcinoma</b>	<b>Gastroesophageal junctional adenocarcinoma</b>	<b>Esophageal squamous-cell carcinoma</b>
<b>Age</b>	↑↑	↑↑	↑↑
<b>Male gender</b>	↑↑	↑↑	↑
<b>Caucasian race</b>	↑	↑	↓
<b>Reflux</b>	↑↑↑	↑↑	--
<b>Barrett's esophagus</b>	↑↑↑	↑↑	--
<b>Obesity</b>	↑↑↑	↑↑	--
<b><i>H pylori</i> infection</b>	↓	↓	?
<b>Achalasia</b>	--	--	↑
<b>Low socioeconomic status</b>	↓	↓	↑
<b>Alcohol</b>	--	--	↑↑
<b>Tobacco</b>	↑	↑	↑↑
<b>Fruit and vegetables</b>	↓	↓	↓
<b>Cereal fiber</b>	↓	↓	--
<b>Fat and meat</b>	↑	↑	--
<b>Hot beverages</b>	↑	↑	↑↑

↑↑↑ Strong positive association; ↑↑ moderate positive association; ↑ weak positive association; ↓ slightly decrease; ? ambiguous studies; -- no association

indicated that green tea is protective.<sup>102-104</sup> The consumption of hot drinks and foods has been suggested as a moderate risk factor for esophageal squamous-cell carcinoma.<sup>101</sup> Thermal injury may cause esophageal cancer via both direct and indirect pathways. Directly, thermal injuries can impair the barrier function of the esophageal epithelium, which may increase the risk of damage from exposure to intraluminal carcinogens. Indirectly, thermal injury could also induce inflammation associated with chronic irritation of the esophageal mucosa by local hyperthermia, and consequently might stimulate the endogenous formation of reactive nitrogen species and nitrosamines.<sup>105</sup> Achalasia is another well-established risk factor for squamous-cell carcinoma by causing mucosa injuries and it causes a 15-fold increased risk of squamous-cell carcinoma.<sup>106-108</sup> Achalasia is a motor disorder of the lower esophageal sphincter, characterized by aperistalsis and failure of the lower esophageal sphincter to relax on swallowing, leading to stagnation of food debris and fluid in a dilated esophagus.<sup>107,108</sup>

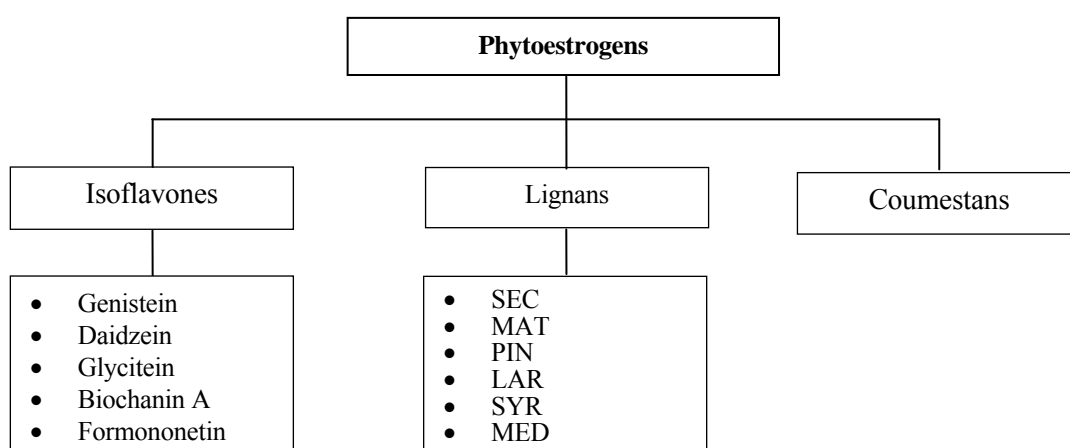
## 2.2 PHYTOESTROGENS AND HUMAN HEALTH

Since phytoestrogen lignans are the main contributors of phytoestrogen intake in Western populations, the main focus of the current thesis is lignans.

## 2.2.1 Classification and food sources

Phytoestrogens are naturally occurring hormone-like compounds found in plant foods which have a unique diphenolic structure. During the 1990s, phytoestrogen-containing food and food supplements with soy or red clover components became increasingly popular as a substitution for the well-established, but controversial pharmacological hormone replacement therapy (HRT). Due to their similarity in chemical structure in human female hormone 17 $\beta$ -estradiol, phytoestrogens have the ability to bind to estrogen receptors (ER) and therefore act as estrogen agonists or antagonists by competing for estradiol and receptor complexes.<sup>109,110</sup> Many epidemiologic and experimental studies have suggested protective effects of phytoestrogens against various estrogen-related diseases, including cardiovascular diseases (CVD),<sup>111,112</sup> breast cancer,<sup>6,113,114</sup> prostate cancer,<sup>8,115</sup> and colon cancer.<sup>9</sup>

Phytoestrogens are subdivided into three main classes: ① isoflavones, ② lignans and ③ coumestans (**Figure 3**).<sup>116</sup> ① Isoflavones are primarily found in soybeans, red clover and other food legumes and nuts.<sup>117</sup> The richest food source soybean contains about 1g of isoflavones per kilogram fresh weight.<sup>118</sup> Genistein, daidzein and glycitein are mainly obtained from soy intake, while red clover contains the isoflavones biochanin A and formononetin.<sup>118</sup> The traditional Asian diet comprises a great amount of soy products, such as tofu, soya milk, and miso soup. In contrast, the Western population rarely consumes soy produces, and hence obtains isoflavones primarily from other legumes, sprouts, and vegetables. Furthermore, in some Western countries, such as Finland and Denmark, soy proteins are added into a variety of foods, e.g. cheese, pasta, salad dressing, hotdogs, meat products and bread. ② Dietary plant lignans constitute the dominant form of phytoestrogen intake in Western populations. They were first detected in humans in 1979 and occur mainly in seeds such as flaxseed, and sesame seed, but in Western society, the major food sources of lignans are whole-grain cereals, beans, other vegetables, some fruits and berries, wines, particularly red wines,<sup>117,119</sup> tea and coffee.<sup>120</sup> Secoisolariciresinol (SEC) and matairesinol (MAT) are two initially identified plant precursors.<sup>121,122</sup> More recently, pinoresinol (PIN), lariciresinol (LAR), syringaresinol (SYR), and medioresinol (MED) were identified. In the Finnish diet, the main sources of SEC are fruit and berries, whereas MAT is mostly derived from whole grains, mainly rye.<sup>123</sup> Whole grain is a rich source of PIN, SYR and LAR, and PIN is also highly present in olive oil.<sup>124,125</sup> ③ Coumestrol, uncommon in the diet, is found in clovers, soy-bean sprouts and in large amounts in mung-bean sprouts.<sup>116</sup>



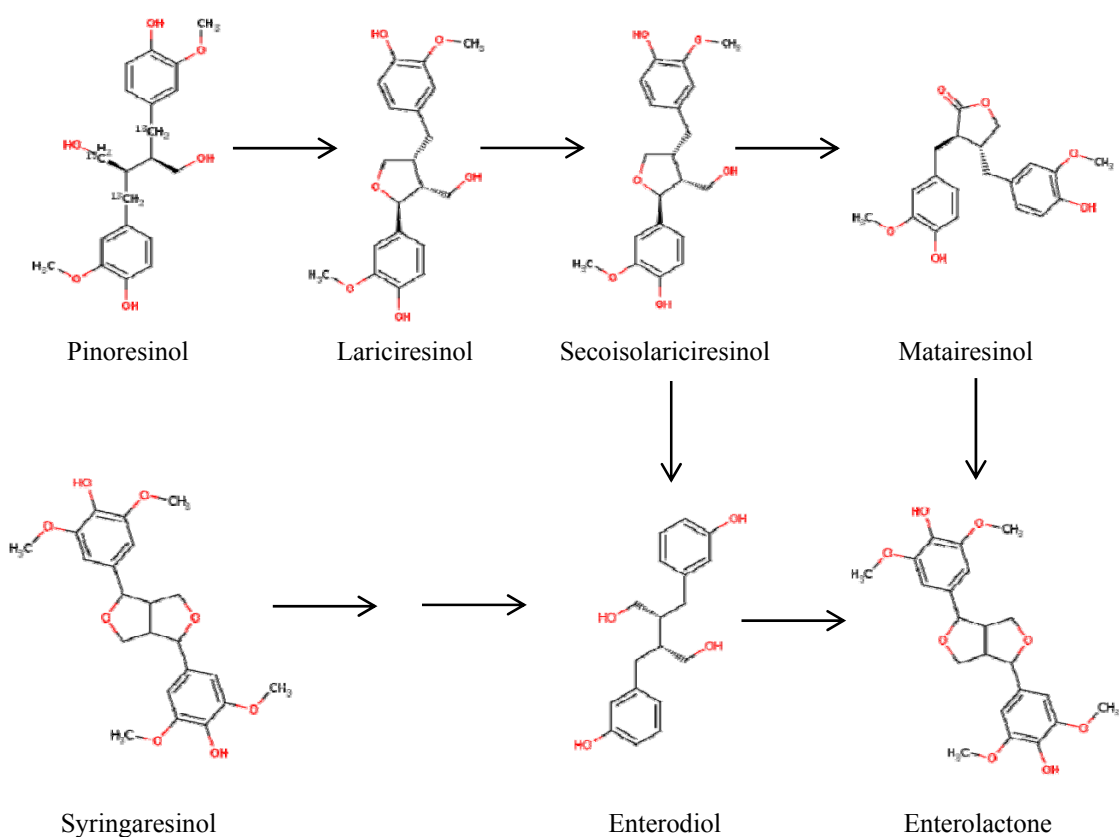
**Figure 3.** Classification of phytoestrogens.

## 2.2.2 Metabolism

### 2.2.2.1 Lignans

After digestion, plant lignans are converted into two major mammalian lignans: enterolactone (ENL) and enterodiol (END), by gut microflora in the proximal colon.<sup>126</sup> The present view of the metabolism of lignans in the gut is presented in **Figure 4**. PIN is converted to LAR and further metabolized to SEC and MAT, which are then converted into ENL and END, respectively. END is oxidized to ENL. SRY is also metabolized into END and then ENL. ENL is the most abundant lignan metabolite in human blood and urine, and its concentration is assumed to reflect recent and habitual intake of dietary plant lignans.<sup>127,128</sup> Serum ENL concentrations and urinary ENL excretion are widely used as biomarkers of plant lignan intake.

Considerable interindividual and intraindividual variations have been observed in various populations.<sup>122,129</sup> It has been suggested that individual variations in the metabolism of plant lignans into mammalian ENL is partly explained by lifestyle and diet.<sup>130</sup> Smoking and high fat intake decrease the plasma ENL, while constipation, intake of whole-grain bread, vegetables, berries and fruits increase the concentration.<sup>130</sup> More importantly, activity of the gut microflora is known as a very influential factor.<sup>131</sup> Therefore, use of antibiotics dramatically decreases the plasma ENL by reducing the amount of colonic bacteria.<sup>132</sup>



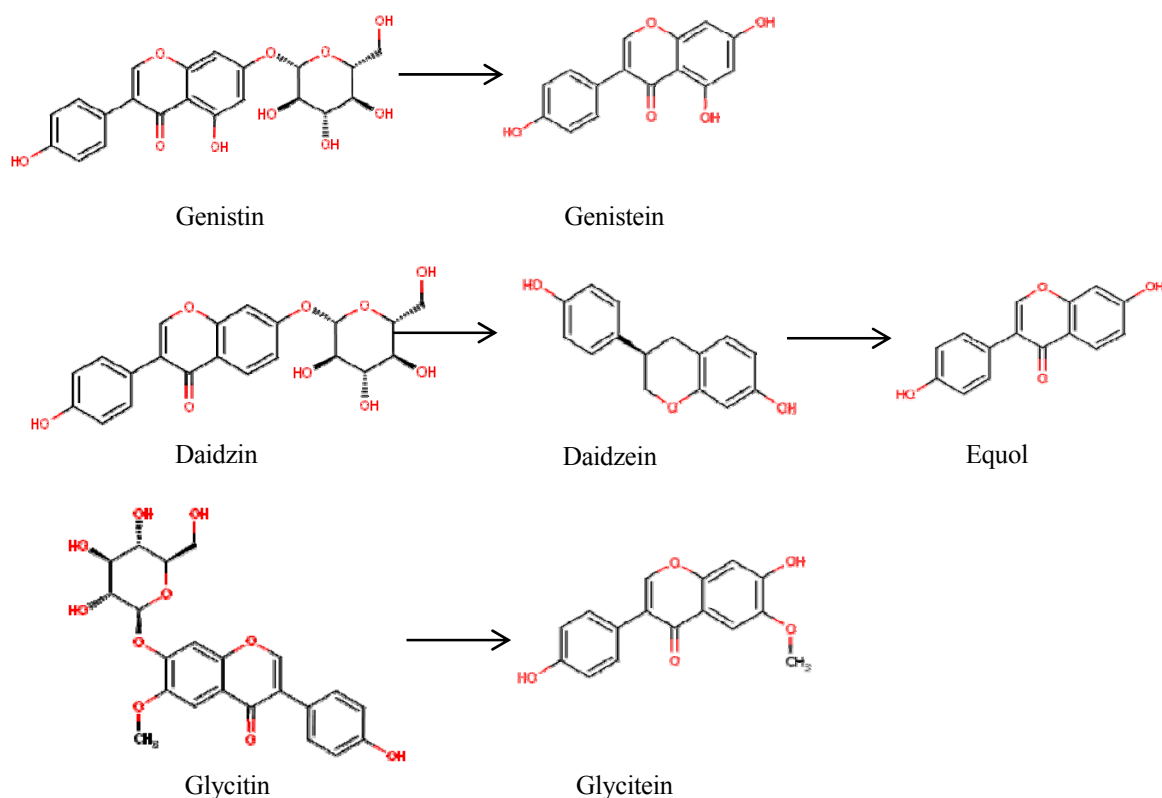
**Figure 4.** Metabolism of plant lignans to END and ENL.<sup>124,126</sup>



### 2.2.2.2 Isoflavone

Isoflavones are present predominantly as glucosides in most commercially available soya products.<sup>133</sup> The major glycosides found in soya beans are daidzin, genistein and glycitin. Upon consumption, these glucose-conjugated compounds are hydrolyzed by mammalian enzyme  $\beta$ -glucosidase or by the gut microflora to form the active isoflavone compounds daidzein, genistein and glycitein (**Figure 5**).<sup>133</sup> Daidzein is further converted by the intestinal microflora into the estrogenic compounds equol and *O*-desmethylangolensin. There is large intra- and inter-individual variation in the level of isoflavone metabolism, and only 23-35% of the individuals in Western populations and 50-55% of Asian populations are able to convert daidzein to equol.<sup>134</sup>

Bioavailability of isoflavone varies among individuals and depends on many factors, including dietary habits,<sup>135</sup> duration of soy consumption,<sup>136</sup> different individual metabolism patterns that might be determined by genetic factors,<sup>137</sup> different bacterial flora,<sup>138,139</sup> and gut transit time.<sup>135</sup> A rapid gut transit time increases bioavailability, whereas a fiber-rich diet decreases the absorption of isoflavones.<sup>135</sup> Equol production is also influenced by diet,<sup>135</sup> showing a higher level of formation in a high carbohydrate environment, but a lower level after use of antibiotics.<sup>140</sup>



**Figure 5.** Metabolism of isoflavones after digestion.<sup>141</sup>

## 2.2.3 Potential mechanisms of action of lignans

### 2.2.3.1 Estrogenic and anti-estrogenic effects

Due to the similar chemical structure to 17- $\beta$  estradiol, mammalian lignan ENL may act as a weak estrogen agonist or antagonist, and numerous studies have demonstrated this biphasic nature of ENL *in vitro* and *in vivo*. These divergent results are difficult to explain, but it has been suggested that the estrogenic or anti-estrogenic effect of lignans depend on the level of endogenous estrogens.<sup>142</sup>

#### 1. Activation of estrogen receptors

The lignan ENL has been shown as a very weak ER agonist and binds to the estrogen receptors, with preference for ER $\alpha$  and very little for ER $\beta$ .<sup>143,144</sup> At a physiological  $\leq 1$   $\mu\text{M}$  concentration, ENL has a stimulatory effect on cell growth on estrogen dependent cell lines MCF-7 and T47D in the absence of estradiol, but in the presence of a slightly stimulatory or non-stimulatory concentration of estradiol, ENL did not cause any stimulation but sometimes a slight inhibition.<sup>145</sup> More specifically, ENL has been observed to mildly increase estrogen-responsive pS2 protein expression.<sup>146</sup>

#### 2. Effects of lignans on steroid metabolizing and other enzymes

Lignans have been shown to inhibit enzymes involved in steroid synthesis, including aromatase, 5- $\alpha$ -reductase, and 17- $\beta$ -hydroxysteroid dehydrogenase. Aromatase, also called estrogen synthetase, is an enzyme responsible for the conversion of androgens to estrogens. ENL has been observed as a moderate inhibitor of placental aromatase, and also in several other cell lines.<sup>147-149</sup> 17- $\beta$ -hydroxysteroid dehydrogenase is the enzyme responsible for the conversion of estrone to estradiol and androstenedione to testosterone. Both ENL and END decrease the activities of aromatase and 17- $\beta$ -hydroxysteroid dehydrogenase in MCF-7 cells, and inhibit the production of estrone and estradiol.<sup>150</sup>

#### 3. Stimulation of sex hormone binding globulin production (SHBG)

It has been observed that urinary ENL in women is positively associated with SHBG, and negatively with the plasma percentage of free estradiol and free testosterone.<sup>151,152</sup> However, it should be noted that this stimulation on SHBG was found at lower physiological concentrations but inhibition at higher.<sup>153</sup> An increase of SHBG will result in a decreased percentage of free or unbound estradiol or testosterone, which are biologically active and are able to enter a cell and activate its receptor.

### 2.2.3.2 Antioxidant activity

ENL and END have shown antioxidant activity *in vitro* only at supraphysiological concentrations.<sup>154,155</sup> In addition, SEC was observed to inhibit the activation of polymorphonuclear leukocytes in a concentration-dependent manner and has nearly five times the antioxidant potency as compared to vitamin E.<sup>156</sup>

### 2.2.3.3 *Anti-obesity activity*

Experimental studies have found that lignans have effects that act against adiposity. ENL has been found to reduce blood lipids and leptin, and induce adiponectin expression in mice,<sup>157</sup> which might result in reduced risk of esophageal cancer. Cell line research has shown that high exposure to leptin is associated with an increased risk of Barrett's esophagus and such exposure can also promote proliferation of EA cells,<sup>158</sup> while high concentrations of adiponectin have shown opposite effects.<sup>159</sup>

### 2.2.3.4 *Inhibition of growth factors*

High plasma levels of insulin-like growth factor I (IGF-I) have been linked to increased risk of several cancer types, including esophageal cancer.<sup>160</sup> *In vivo*, an intake of flaxseed or purified SEC diglycoside in rats reduced plasma concentration of IGF-I.<sup>161</sup> ENL was also detected to inhibit IGF-I signaling in several cancer cells, such as prostate cancer and breast cancer.<sup>162,163</sup>

## 2.2.4 Lignans and gastrointestinal cancer

Although experimental studies have demonstrated a protective role of phytoestrogen lignans in the development of gastrointestinal cancer, limited evidence is available from observational studies (**Table 2**).

### **Esophageal cancer**

Esophageal adenocarcinoma and gastroesophageal junctional adenocarcinoma are characterized by a strong male predominance that remains to be explained.<sup>21</sup> Estrogens have been suggested to contribute to this pattern.<sup>83</sup> The beneficial effects of lignans on esophageal and gastroesophageal junctional adenocarcinoma might be attributed to their potential estrogenic property. The estrogenic effects of lignans are widely assumed to be mediated by the mammalian metabolite ENL and via activating ERs.<sup>164</sup> Evidence from previous studies suggest that the protective effect of estrogen on esophageal cancer is mediated by the ERs.<sup>165</sup> Since the presence of ERs has been identified in esophageal tissue,<sup>166,167</sup> a linkage between mammalian lignans and esophageal cancer via ERs is possible. In addition to their potential estrogenic properties, lignans have been demonstrated to attenuate high-fat diet-induced fat accumulation and influence serum levels of adipose tissue related hormones, such as leptin and adiponectin.<sup>157</sup> Cell line research has shown that high exposure to leptin is associated with an increased risk of Barrett's esophagus and such exposure can also promote proliferation of esophageal adenocarcinoma cells,<sup>158</sup> while high concentrations of adiponectin have shown opposite effects.<sup>168</sup> ENL has been found to reduce blood lipids and leptin, and induce adiponectin expression in mice.<sup>157</sup>

## ***Colorectal cancer***

In 1984, Adlercreutz suggested that lignan intake might be protective with regard to colon cancer.<sup>169</sup> Since then, the majority of studies investigating the role of lignans in colon carcinogenesis have been either in vitro or animal study. A recent study observed that at 100 microM concentration, both ENL and END significantly reduced the proliferation of all four studied colon cell lines (LS174T, Caco-2, HCT-15, T-84).<sup>170</sup> Moreover, lignan-rich foods including flaxseed and rye bran were also observed to protect against colon cancer or formation of colon polyps.<sup>171,172</sup>

To date, only 6 studies have investigated the potential effect of plant lignans,<sup>173-175</sup> or mammalian enterolignans,<sup>9,176,177</sup> in the development of colon cancer. All three studies addressing lignan intake exposure indicated lignans' beneficial role. Heather W. *et al.* calculated the enterolignans intake from animal origin such as meat, seafood and nonmilk dairy and reported that high intake of ENL and total enterolignans (ENL, equol) was associated with reduced risk of colon cancer by approximately 70% among women.<sup>175</sup> Although the plant lignan intake seemed to be protective, evidence based on a biomarker of enterolignans did not fully support this potential function. In a prospective study, the overall relationship between plasma enterolignans and colorectal cancer in the full study was null, but there was evidence of increased risk among particular subgroups: current smokers, overweight, and women, particularly postmenopausal women.<sup>177</sup> In contrast, a case-control study reported that higher concentration of plasma ENL was associated with lower risk of colon cancer among women but higher risk of rectal cancer among men.<sup>9</sup>

## **Gastric cancer**

A prospective study using data from EPIC was the only available study evaluating lignan intake and gastric cancer. During an average follow-up of 11 years, 683 incident cases of gastric cancer were identified.<sup>178</sup> The mean intakes of lignans were 1640 µg/day and 1400 µg/day for men and women, respectively. No statistically significant association was found for lignan intake and gastric cancer risk.

**Table 2.** Summary of studies investigating lignan exposure in relation to risk of colon and gastric cancer.

Study	Study design	Cases/ total cohort or controls	Exposure measurement	OR/RR/IRR (95% CI)*	Adjustment
<b>Colon cancer</b>					
Michelle Cotterchio (2006)/USA <sup>173</sup>	population-based case-control	1095/1890	<i>FFQ</i> <i>Intake:</i> plant lignans (SECO, MAT): T3 (>1338 µg /day) vs. T1( 0-531 µg /day)	0.73 (0.56, 0.94)	Age, sex, and total energy intake.
Heather Ward (2010)/UK <sup>175</sup>	nested case-control	221/886	7-day diet diaries; <i>Intake:</i> 1) plant lignans (SECO, MAT): continuous value 2) enterolignans (ENL, equol): continuous value	<b>Men:</b> plant lignans: 1.07 (0.73, 1.57); enterolignans 0.94 (0.53, 1.67); ENL: 1.00 (0.66, 1.66) <b>Women:</b> plant lignans: 1.52 (0.88, 2.64); enterolignans 0.32 (0.13, 0.79); ENL: 0.33 (0.14, 0.74)	Age, height, weight, family history of colorectal cancer, smoking status, aspirin use, physical activity, and average daily intake of fat, energy, calcium, fiber, alcohol, and red and processed meats.
Raul Zamora-Ros (2012)/Spain <sup>174</sup>	hospital-based case-control	424/401	600-item FFQ; <i>Intake:</i> plant lignans (SECO, MAT, LAR, PIN): Q4 (> 500 µg/ 1,000 kal day) vs. Q1(<270 µg/ 1,000 kal day)	0.52 (0.28-0.96)	Age, sex, BMI, energy intake, alcohol and fiber intake, red and processed meat intake, tobacco consumption, physical activity, regular drugs (aspirin, non-steroidal anti-inflammatory drug, both, none), and family history of colorectal cancer.
Heather Ward (2008)/UK <sup>176</sup>	nested case-control	221/889	<i>Serum and urine</i> END, ENL: continuous value	<b>Serum</b> END: 1.04 (0.99-1.09); ENL: 1.02 (0.95-1.10); Total lignans: 1.03 (0.94-1.12) <b>Urine</b> END: 1.04 (0.99-1.09); ENL: 1.02 (0.95-1.10); Total lignans: 1.03 (0.94-1.12)	Age, sex, height, weight, and average intake of fiber and calcium.
Anneleen Kuijsten (2008)/Netherland <sup>177</sup>	nested case-control	160/387	<i>Plasma</i> END: Q4 (≥ 1.96 nmol/L) vs. Q1 (<0.45 nmol/L); ENL: Q4 (≥ 18.7 nmol/L) vs. Q1 (<4.11 nmol/L);	END: 1.11(0.56-2.20) ENL: 1.70 (0.88-3.27)	Age, sex, study center, BMI, alcohol, smoking status, duration of smoking, physical activity during leisure time and at work, aspirin use, education level, intake of energy, fiber, calcium, meat, fish, fruit, vegetables, tea, wine, and whole grain bread.
Nina Føns Johnsen (2010)/Denmark <sup>9</sup>	nested case-control	244/370	<i>Plasma</i> ENL: continuous values	<b>Colon cancer</b> Women: 0.73 (0.56-0.94) Men: 1.19 (0.93-1.51); <b>Rectal cancer</b>	Years of education, baseline values of present use of hormone replacement therapy, use of non-steroidal anti-inflammatory drugs, BMI, present smoking, bowel movements per week, intake of

Women: 0.83 (0.57-1.20)  
Men: 1.74 (1.25-2.44);

red and processed meat, alcohol and whole grain products.

***Gastric cancer***

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Zamora-Ros R (2012)/ Europe <sup>178</sup>	Cohort study	683/477,312	<i>FFQ</i> Plant lignans ( <i>SECO, MAT, LAR, PIN, ENL, ENT</i> ) <i>Men: Q4 (&gt;2.1 mg/day) vs. Q1 (&lt;1.0 mg/day)</i> <i>Women: Q4 (&gt;1.7 mg/day) vs. Q1 (&lt;0.9 mg/day)</i>	<b><i>Men:</i></b> 0.99 (0.63, 1.55)  <b><i>Women:</i></b> 0.94 (0.54, 1.64)	Age, sex, center, education level, smoking status, physical activity, BMI, alcohol and energy intake, and daily consumption of fruit, vegetables, and red and processed meat.
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\* OR: odd ratio; RR: relative risk; IRR: incidence rate ratio

## 2.3 QUERCETIN, RESVERATROL AND HUMAN HEALTH

### Quercetin

Quercetin is a flavonol found in a variety of human foods such as onions, grapes, apples, berries, cherries, broccoli, citrus fruits and tea. Quercetin is known for its wide range of biological effects such as anti-oxidative effects,<sup>179</sup> increasing lipolysis,<sup>180</sup> inducing apoptosis<sup>181</sup> as demonstrated by animal studies. The idea that quercetin might also exert anti-cancer effects has also been confirmed by *in vitro* and *in vivo* studies.<sup>181,182</sup>

### Resveratrol

Resveratrol is a naturally occurring polyphenol found mainly in grapes, a variety of berries, peanut, and medical plants. The most important dietary source of resveratrol is red wine, and the containing resveratrol is usually postulated to be the important factor for the beneficial effect of red wine for human health.<sup>183</sup> Resveratrol has received considerable attention since 1997 for its various pharmacological effects, including anti-oxidant, anti-cancer, anti-inflammatory and anti-platelet properties.<sup>184-186</sup> It has also been shown to reduce the synthesis of lipids by repression of PPAR $\gamma$  in differentiate adipocytes, suppress proliferation and induce apoptosis.<sup>187-189</sup>

## 2.4 SYNERGISTIC EFFECTS BETWEEN PHYTOCHEMICALS

Quercetin, resveratrol and lignans are the most widely studied phytochemicals in Western population due to their potential beneficial effect on human health. Quercetin has been reported to increase lipolysis in rat adipocytes and induction of apoptosis.<sup>180,190</sup> Resveratrol has also been shown to reduce the synthesis of lipids in rat liver and play an important role in suppressing proliferation, and inducing apoptosis in hematopoietic cells *in vitro*.<sup>189</sup> Interestingly, the combined intakes of phytoestrogen isoflavone, quercetin and resveratrol were observed with synergistic effect in suppression of adipogenesis and induction of apoptosis in an animal study.<sup>191</sup> These evidences provide a valid rationale to evaluate the beneficial role of total intake of these phytochemicals in the development of esophageal cancer. Since isoflavone is the major phytoestrogen source in Asian diet, while lignan is the dominant phytoestrogen intake among Western population, the current thesis will focus on lignan intake instead of isoflavone.

### 3 AIMS OF THE STUDIES

Based on the evidence from experimental studies, dietary intake of phytoestrogens lignans and in combination with other phytochemicals quercetin and resveratrol could protect against esophageal cancer. However, none of the current epidemiological studies has demonstrated this possible phytoestrogen-esophageal cancer relation. In this thesis, we aimed mainly to investigate whether dietary intake of phytoestrogen lignans could influence the risk of esophageal cancer in the Swedish population.

**Paper I** To determine the association between dietary intake of lignans and risk of esophageal cancer in a Swedish population-based case-control study.

**Paper II** To validate the use of a food frequency questionnaire for the assessment of dietary intake of lignans compared to the serum biomarker enterolactone.

**Paper III** To clarify whether dietary intake of lignans is associated with the risk of esophageal and gastric adenocarcinoma using a prospective design.

**Paper IV** To define a food pattern characterized by high intake of the phytochemicals lignans, quercetin and resveratrol, and to evaluate the association of this food pattern in relation to risk of esophageal cancer.



## 4 MATERIAL AND METHODS

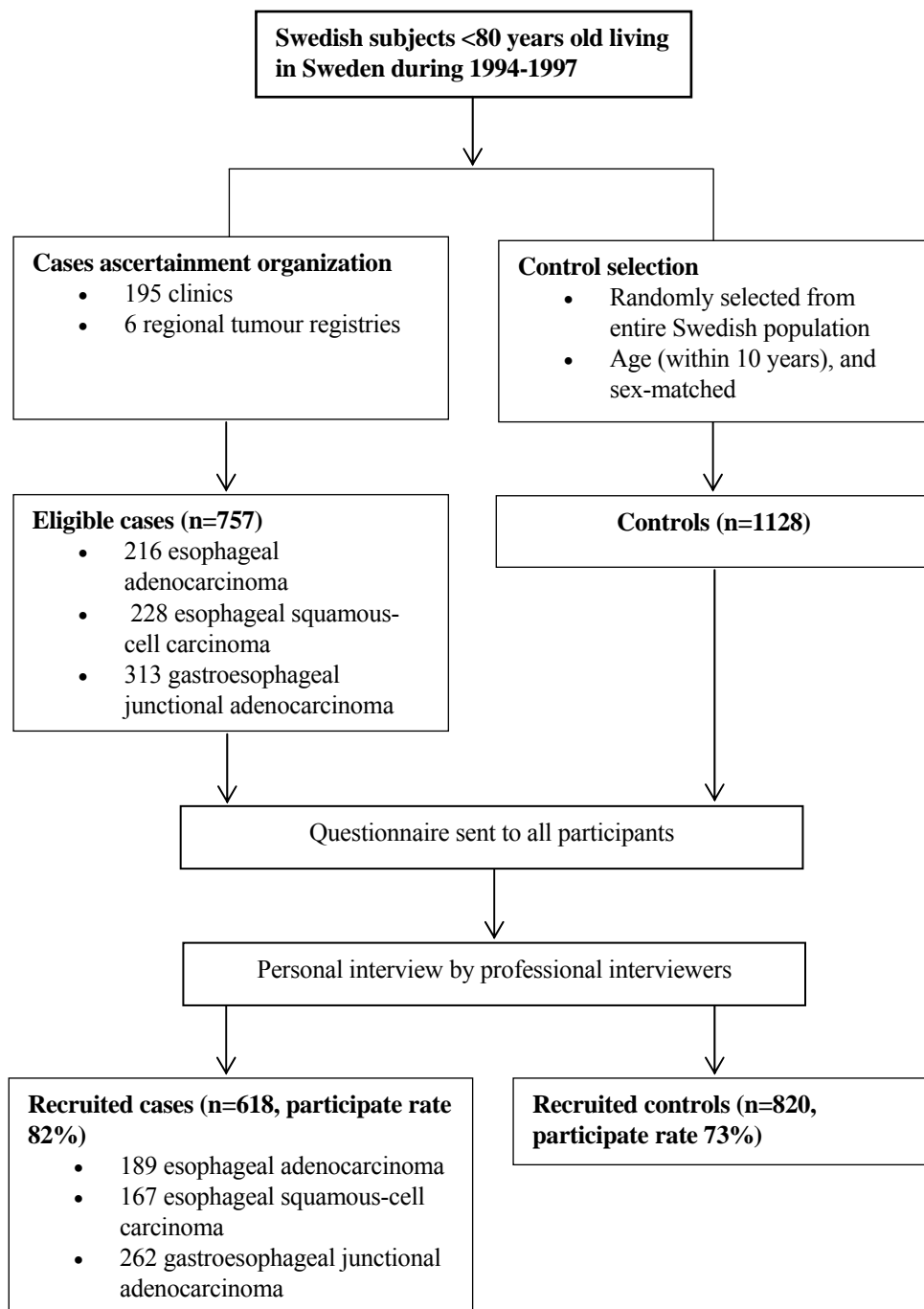
### 4.1 SUBJECTS AND STUDY DESIGN

#### *The Swedish Esophageal and Cardia Cancer Study*

The nationwide Swedish Esophageal and Cardia Cancer study (SECC)<sup>38</sup> was a population-based case-control study that enrolled subjects younger than 80 years, born and living in Sweden from December 1, 1994, through December 31, 1997 (**Figure 6**). All newly diagnosed patients with esophageal or gastroesophageal junctional adenocarcinoma, and half of those with esophageal squamous cell carcinoma (those patients born on even-numbered days) were eligible as cases. The reasons for reducing the number of esophageal squamous cell carcinoma were three: 1) The main objective of the study was to investigate risk factors of the adenocarcinomas, 2) The incidence of squamous cell carcinoma was higher than that of adenocarcinoma during the inclusion period, and 3) Cost-efficiency reasons. The study included extensive personal interviews with all participants.

A comprehensive organization for the rapid ascertainment of cases was used to ensure that every potential case patient throughout the country was identified soon after diagnosis. The organization included contacting persons at all 195 departments of general surgery, thoracic surgery, otorhinolaryngology, oncology, and pathology in Sweden, as well as continuous collaboration with the six regional tumor registries. Age- (within 10 years) and sex-matched controls were selected randomly by Statistics Sweden from the subjects in the entire Swedish population using the Swedish Register of Total Population. The numbers of controls selected in each defined stratum were adjusted to approximate the age and sex distribution of the patients with esophageal adenocarcinoma. All cases and controls were personally interviewed by professional interviewers from Statistics Sweden, who were taught to treat cases and controls in a strictly similar manner. The participants were asked about background data and exposure to established etiological factors, which have been addressed in detail in separate publications, including gastroesophageal reflux,<sup>38</sup> obesity,<sup>44</sup> tobacco smoking,<sup>53</sup> alcohol drinking,<sup>53</sup> education,<sup>192</sup> physical activity,<sup>44</sup> childbearing,<sup>193</sup> and *Helicobacter pylori* infection.<sup>194</sup> Data on infection with *Helicobacter pylori* was assessed by serology from blood samples, which were collected from a majority of the cases and controls, as presented in detail elsewhere.<sup>194</sup> The SECC database was used for **Paper I** and **IV** of the thesis.

**Figure 6.** Study population for Paper I and IV.



### ***The Swedish Mammography Cohort***

The Swedish Mammography Cohort (SMC) was established between 1987 and 1990, when all women born between 1914 and 1948 residing in two counties in central Sweden Västmanland (n=41,786) and Uppsala counties (n=48,517) were invited to participate in a population-based mammography screening program (**Figure 7**). The invitation letter was accompanied by a questionnaire (the FFQ-87 with 67 food items) that asked for information on dietary factors, weight, height, education level, and

family history of cancer.<sup>195</sup> A total of 66,651 women (74% of all eligible) returned a completed questionnaire. From the baseline cohort, we excluded participants with incorrect or missing personal identity numbers, missing date on the questionnaire, date of migration, or date of death. Additional exclusion was also applied for women who had previous cancer diagnosis, consumed implausibly low or high energy intake, and who died or moved out of the study area between baseline and autumn 1997. A total number of 10,621 women were excluded based on the above criteria. In the autumn of 1997, an expanded questionnaire (the FFQ-97 with 93 food items) with about 350 items concerning diet, other lifestyle factors, and medical history was mailed to all women who were still alive and residing in the study area (n=56,030). Among them, 39,227 women (70% of eligible) completed the questionnaire, 2530 of whom were further excluded due to incorrect personal identity number, implausible energy intake and cancer diagnosis before January 1998. The FFQ-97 elicited information on weight, height, waist and hip circumferences, diet, and lifestyle factors.

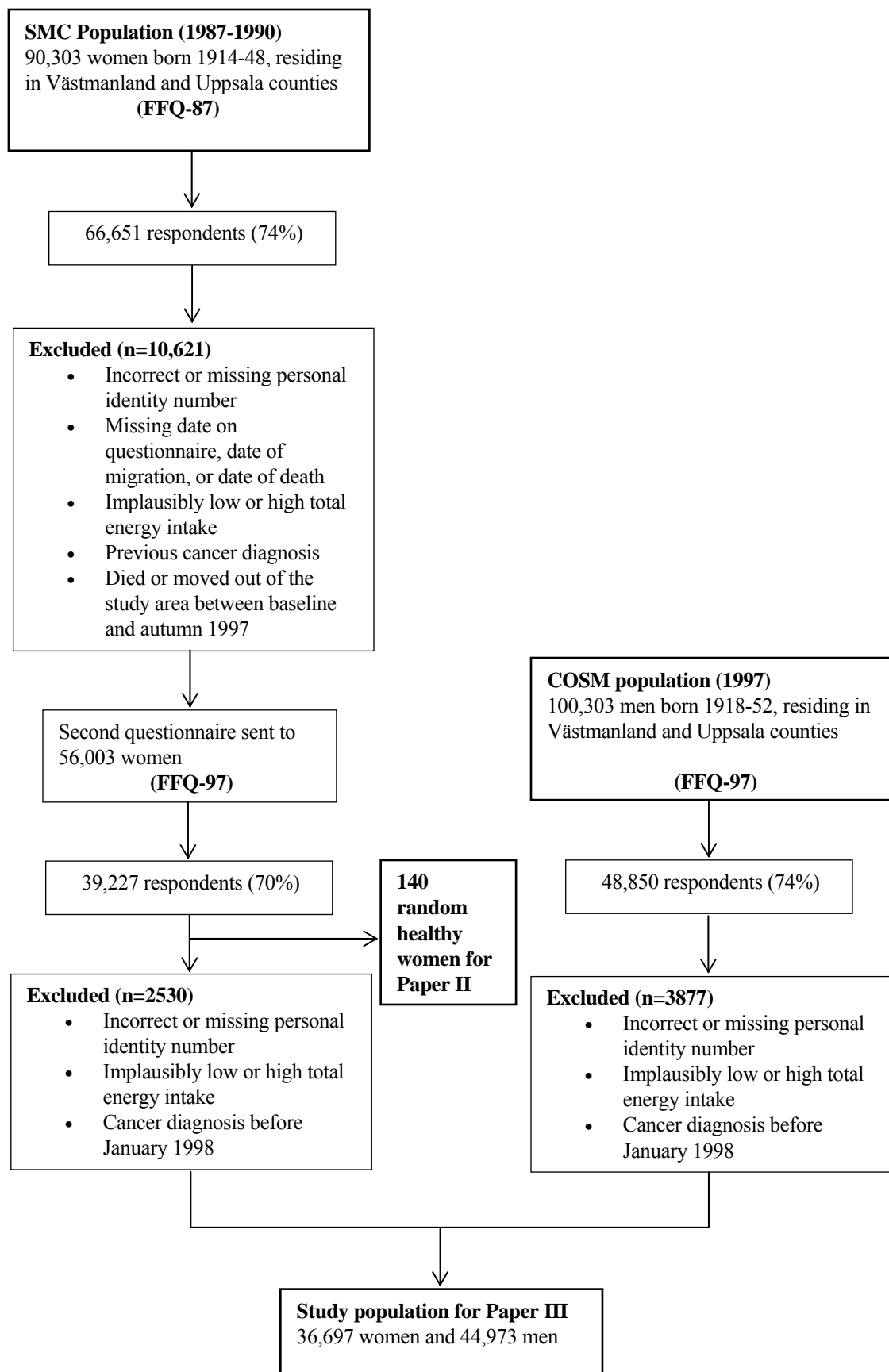
During 2003-2004, 140 women aged 55 to 75 years were randomly selected from the SMC for **Paper II (Figure 7)**. None of them had used antibiotics in the past year. This exclusion was necessary since antibiotics are known to influence phytoestrogens' metabolism. All 140 participants answered the FFQ-87 and the FFQ-97 in 2003-2004, and fasting blood samples were collected within 3 months of completing these questionnaires. The information on history of gastrointestinal disease was obtained from the National Patient Register, while the diabetes data was collected from the combination of the National Patient Register, the National Diabetes Register and self-reported questionnaires.

### ***The Cohort of Swedish Men***

The Cohort of Swedish Men (COSM) began in the autumn of 1997, when 48,850 men (49% of all eligible) born between 1918 and 1952 and residing in two counties in central Sweden (Västmanland and Örebro counties) answered a mailed questionnaire that was identical (except for some sex-specific questions) to the SMC questionnaire in 1997 (FFQ-97) (**Figure 7**). Men (n=3877) with wrong or missing personal identity numbers, those with implausibly low or high total energy intake (i.e., three standard deviations from the mean value) and those with cancer (except non-melanoma skin cancer) diagnosed before enrollment or during the first year of follow-up were excluded.

Eligible participants for **Paper III** were those women and men who completed the 1997 questionnaire after exclusion, leaving 36,697 women from SMC and 44,973 men from COSM for the analysis.

**Figure 7.** Study population for Paper II and III.



## 4.2 EXPOSURE MEASUREMENT

### 4.2.1 Dietary assessment of lignans and other nutrients intake

#### *Paper I & IV: 63-item FFQ*

Dietary habits were assessed by use of a written validated FFQ concerning the habitual intake of 63 foods and beverages.<sup>196</sup> The questionnaires were distributed before the face-to-face interviews with professionals from Statistics Sweden, and the given answers were checked during the interview. Missing answers or other uncertainties in questionnaires were as far as possible completed and clarified during these interviews. The FFQ examined dietary habits 20 years before the interview, with the purpose of obtaining a plausible induction time between the exposure and the invasive cancer. The frequency of intake of each food item was assessed on the basis of open answers, i.e., number of times of consumption per day, week, month or year. A detailed list of food items stratified by 26 food groups is shown in **Table 3**.<sup>197</sup>

The calculation of lignan intake included six precursors of mammalian lignans: MAT, SECO, LAR, PIN, SYR, and MED. To calculate the intake of specific lignans, we categorized the contents of all six precursors in various dietary items based on published analytical data.<sup>116-118,126,198-202</sup> When multiple values were reported in the literature, averages were calculated; when values were given in ranges, midpoint values were used. The contents of the lignans LAR, PIN, SYR, and MED in different grain flours were used to estimate lignan content of bread and cereal products. Different contents of quercetin from onions, broccoli, lettuce, tomatoes, strawberries, apples, wine, tea, and fruit juice were accumulated.<sup>203-206</sup> The major sources of resveratrol were derived from intake of wine, tea, and berries.<sup>206-209</sup> Energy contents were obtained from the Swedish National Food Administration database.<sup>210</sup> The exposure of dietary lignans was expressed in nutrient density by dividing the estimated daily intake of lignans ( $\mu\text{g}/\text{day}$ ) by the estimated total energy intake ( $\text{MJ}/\text{day}$ ) according to a Multivariate Nutrient Density Model.<sup>211</sup> While calculating each food item intake, the frequency of consumption was multiplied by sex-specific portion size, using data from the Swedish national diet survey (Riksmaten 1997-1998).<sup>212</sup>

**Table 3.** List of food items in food frequency questionnaire used in paper I and IV.<sup>197</sup>

<b>Food groups</b>	<b>Items</b>
Vegetables	Green salad, cauliflower, white cabbage, red cabbage, spinach, carrot, onion, leek, garlic
Tomatoes	Tomatoes
Fruit	Citrus fruits, apple, pear, banana, plums
Whole grains	Whole grain bread, crisp bread, soft bread, oatmeal, bran
Refined grains	Rice, macaroni, spaghetti, pancakes, waffles
Cereal	Porridge (oatmeal, rice, corn flour, semolina), infant cereal, cereal/muesli

Low-fat dairy	Low fat milk
High-fat dairy	Whole milk, milk powder, cheese, cream, ice cream
Dressing or sauce	Bechamel sauce, béarnaise sauce
Fish	Herring, cod, flat, hake, whiting, haddock, turbot, salmon, white fish, eel, pike, perch
Poultry	Chicken
Red meat	Meat ball, pork, beef, liver, minced meat
Processed meat	Smoked salted pork, “Falu” sausages, hamburgers, hot dogs, blood sausages
Eggs	Fried eggs, boiled eggs, omelettes
Potatoes	Boiled potatoes, baked potatoes
French fries	French fries, fried potatoes
Sweets	Buns, Danish pastries, cookies, sweet biscuits, tarts, cakes, chocolate, jam, marmalade, fruit sauce
Juice	Fruit juice
High energy drinks	Fruit syrup, soft drinks
Nuts	Nuts
Tea	Tea
Coffee	Coffee
Beer	Light beer, median beer, strong beer
Wine	Red wine, white wine
Liquor	Brandy, gin, vodka, rum, whiskey, liqueur

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***Paper II & III: FFQ-87 & FFQ-97***

The FFQ-87 and the FFQ-97 included 67 and 93 food items, respectively. In the FFQ-87, participants were asked to report their average frequency of consumption of each type of food or beverage using 8 predefined frequency categories: ‘never/seldom’, ‘1-3 times/month’, ‘1 time/week’, ‘2-3 times/week’, ‘4-6 times/week’, ‘1 time/day’, ‘2-3 times/day’, or ‘4 times/day’. In the FFQ-97, close-ended questions with similar response categories were set for most food items, but open-ended questions (open answers, not pre-specified categories) were designed for some commonly consumed foods including bread, milk, cheese, soft drinks, beer, coffee, tea and sugar. Total lignan intake was estimated by summing up all six of the most prevalent dietary precursors of ENL: SEC, MAT, LAR, PIN, MED, and SYR. Of the 67 and 93 food items listed in the FFQ-87 and the FFQ-97, 45 (69.2%) and 65 (69.9%) items were assigned lignan values, respectively. The remaining had no values assigned because the lignan content was assumed to be negligible. Nutrient intake was computed by multiplying the frequency of food items by the nutrient content of the age-specific servings. The estimations of total intake of lignans were adjusted for total energy intake, using the Residual method.<sup>213</sup> Since the activity of the gut microflora influences the metabolism of dietary lignans to ENL, we also used a formula based on experimental results to calculate the expected amount of mammalian lignan ENL in

**Paper II:**<sup>214</sup>

$$\text{ENL} = 0.62 * \text{MAT} + 0.72 * \text{SEC} + 1.01 * \text{LAR} + 0.55 * \text{PIN} + 0.04 * \text{SYR} + 0.8 * \text{MED}.$$

#### 4.2.2 Analysis of serum enterolactone

Blood samples were processed and separated for sera that were stored at  $-80^{\circ}\text{C}$  until analysis. Samples were shipped frozen to the Folkhälsan Institute for Preventive Medicine, Nutrition and Cancer (Helsinki, Finland), where they were thawed and subjected to overnight enzymatic hydrolysis and ether extraction. Sample extracts were then diluted in assay buffer, with europium-label, internal standards, and subsequently analyzed by time-resolved fluoroimmunoassay (TR-FIA) according to previously reported protocols for assessment of ENL.<sup>215-217</sup> The intra- and inter-assay coefficient of variation (CV %) of the TR-FIA method was low (3.3-6.0% and 6.9-9.9% for ENL, depending upon the serum concentrations).<sup>215-217</sup> Serum isoflavone genistein was analyzed using the same method, but only 40 of the total 140 women had a detectable serum concentration within the range 0-48 nmol/L (median=3 nmol/L), indicating low consumption of isoflavone genistein. Therefore, genistein was not included in the final analysis.

#### 4.2.3 Lifestyle factors and other potential confounders

In addition to dietary habit, the questionnaires also covered various lifestyle indicators including e.g. BMI, smoking status, physical activity, education level, and reflux symptoms. BMI was calculated as body weight divided by square of the body height in meters ( $\text{kg}/\text{m}^2$ ), and classified into 4 subgroups:  $<18.5$ , 18.5-24.9, 25-29.9, or  $\geq 30$   $\text{kg}/\text{m}^2$  in **Paper I** and **Paper IV**, 3 subgroups:  $<25$ , 25-29.9, or  $\geq 30$   $\text{kg}/\text{m}^2$  in **Paper II** and **III**. The participants with a BMI of 25-29.9  $\text{kg}/\text{m}^2$  were considered as overweight, while a BMI  $\geq 30$   $\text{kg}/\text{m}^2$  represented obesity. Smoking habit was classified into: nonsmoker, past smoker, or current smoker. Physical activity was categorized into four levels (I-low, II, III, IV-high), using a combination of 12 variables, reflecting the physical activity level during both leisure time and at work. Heartburn and acid regurgitation were considered to be the symptom of gastroesophageal reflux (yes/no). Participations were also asked for the duration of years of education, which was classified into 3 levels:  $\leq 9$ , 10-12, and  $\geq 13$  years.

**Paper I** and **IV**: Data on infection with *Helicobacter pylori* were assessed by serology from blood samples, which were collected from the majority of the cases and controls, as presented in detail elsewhere.<sup>89</sup>

**Paper II**: The information on history of gastrointestinal disease was obtained from the National Patient Register, while the diabetes data was collected from the combination of the National Patient Register, the National Diabetes Register and self-reported questionnaires.

### 4.3 CASE ASCERTAINMENT

#### **Paper I and IV**

All newly diagnosed patients with esophageal or gastroesophageal junctional adenocarcinoma and half of those with esophageal squamous cell carcinoma were

eligible as cases. Uniform routines for tumor classification were introduced at all participant sites. A comprehensive organization for rapid ascertainment of cases included collaboration with 195 hospital departments and the six regional tumor registries. The tumor classification was completed as follows:

- 1) All patients underwent esophago-gastroscopy. The gastroesophageal junction was defined as the point where the proximal longitudinal mucosal folds begin in the stomach.
- 2) Serial biopsies were performed according to a special protocol in order to obtain a correct histo-pathological diagnosis and to determine the exact location of the tumor.
- 3) Surgeons and the pathologists provided detailed descriptions of the location of the cancer on special forms.
- 4) Almost all biopsies and surgical specimens were finally re-examined by a single experienced pathologist (Anders Lindgren).
- 5) For those ambiguous cases, a panel of surgeons and pathologists classified the cases, using all available information.

### **Paper III**

All newly diagnosed cases of esophageal and gastric adenocarcinoma were identified through linkage to the Swedish Cancer Register, which was established in 1958 and has been estimated to be highly complete. The completeness of the registry was 63% for esophageal adenocarcinoma, 74% for cardia adenocarcinoma, and 98% for gastric cancer.<sup>218,219</sup> Ascertainment of death date was accomplished by linkage to the Swedish Causes of Death Register and the Swedish Register of the Total Population. Cases of esophageal or gastric adenocarcinoma were defined as the first diagnosed malignant neoplasm detected since entry into the cohort. We included only cases of adenocarcinoma since this is the main histologic type of malignancy in the upper gastrointestinal tract and the etiology of adenocarcinomas is different from other histological types. Moreover, other histological types of malignant esophageal or gastric tumors do not share the unexplained male predominance in incidence. Each participant was followed from January 1, 1998 until the date of diagnosis of adenocarcinoma of the esophagus or stomach, any other cancer, death, or end of study period (December 31, 2009), whichever came first. To avoid detection bias, we excluded all persons-years during the first year of follow-up and all participants diagnosed with any cancer during the first year of follow-up. Due to the similar etiology and pattern of incidence, gastroesophageal junctional adenocarcinoma was combined with esophageal adenocarcinoma, while gastric adenocarcinoma was analyzed separately.

## **4.4 STATISTICAL ANALYSIS**

### **Paper I and IV**

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). The exposure was categorized into four levels based on the



distribution among the controls, with about an equal number of controls in each quartile. The lowest quartile of intake density was set as the reference. Possible confounding or effect modification by all established etiologic factors were considered in the analyses: age (<55 years, 55-64 years, 65-74 years, or  $\geq 75$  years), sex, education ( $\leq 9$  years, 10-12 years, or  $\geq 13$  years), BMI (<18.5, 18.5-24.9, 25-29.9, or  $\geq 30$  kg/m<sup>2</sup>), tobacco smoking status (never smoker, previous smoker, or current smoker of any tobacco), alcohol consumption (no alcohol, >0-15 g/week, 16-70 g/week, or >70 g/week), physical activity (in quartiles), gastroesophageal reflux symptoms (yes or no), and *Helicobacter pylori* infection (yes or no). A basic model included adjustment for age, sex, and energy intake only, while the full multivariate model included all variables listed above, selected depending on the specific tumor studied.

In **paper I**, sex-stratified analyses were also performed since the effect of estrogen was hypothesized to be sex specific. The variable childbearing was not included in the full model for women since non-significant effect was detected. Sensitivity analysis was used to identify whether any missing values from those food items with extremely high lignan content, i.e. white bread, whole grain bread or crisp bread, could influence the association between intake of lignans and the studied malignancies. Since there were some missing values in the food items, we analyzed the data in two ways: 1) all the missing values were kept as a separate category, and 2) exclusion of participants with more than 10% missing values. Among these excluded participants, the distributions of missing data were similar between different gender and age groups. Since the results were similar with or without exclusion, we reported only the results with exclusion.

In **paper IV**, to derive a dietary pattern defining intake of lignans, quercetin and resveratrol, the statistical method RRR was employed using the partial least squares procedure in SAS.<sup>220</sup> The number of extracted pattern scores equals the number of response variables, i.e. three pattern scores were extracted, one for each single phytochemical related dietary pattern. The first pattern score was retained for the subsequent analyses because it explains most of the variation for three phytochemicals intakes. Total intake of all phytochemicals was analyzed in energy adjusted densities, i.e., per 1,000 kcal/day. To conduct less population-dependent pattern variables, the original dietary pattern scores were shortened and simplified.<sup>221</sup> The simplified pattern scores were obtained by summing unweighted standardized scores of intake for each food group, which explains most of the inter-individual variation in the original dietary pattern scores. We applied the commonly used simplified approach by selecting only food items with high factor loadings  $\geq |0.2|$ .<sup>221</sup> Factor loadings represent the correlations of each food item intake with the dietary pattern score. The simplified pattern scores were used to evaluate the association between the pattern and the risk of esophageal cancer subtypes in logistic regression models. The level of food pattern score was categorized into quintiles based on the distribution among the control participants. To test for linear trend across dietary pattern scores, we used the median scores within each quintile, and treated these values as a continuous variable.

## **Paper II**

Lignan values were not normally distributed; therefore the log-transformed values of lignan intake and serum ENL were used in the Pearson's correlation analyses. A total of five observations outside the 95% CIs of the corresponding values were excluded, leaving 135 participants for final analysis. The following variables were considered as potential confounders since they might influence the metabolism of dietary lignans: age (categorized into three groups: 55-61, 62-69, or >69 years), BMI ( $\leq 24.9$ , 25-29.9, or  $\geq 30$  kg/m<sup>2</sup>), constipation (yes or no), gastrointestinal disease history (yes or no), and diabetes (yes or no). The analyses were implemented in both crude and multivariable models. The basic model included adjustment for age only, while the full multivariable model included all variables listed above. The partial Pearson's correlation was used to calculate the adjusted correlation coefficients in the full model. Test of linear trend across BMI categories was conducted by assigning the median of BMI in each BMI category and then treating these values as a continuous variable in the model. Additional analyses stratified by BMI subgroups were also conducted. Analysis of variance (ANOVA) and t-test were used to compare the mean values of serum concentration of ENL in the subgroups when appropriate.

## **Paper III**

Hazard ratios (HRs) and 95% CIs were computed using Cox proportional hazard models, with follow-up person-days as the underlying time metric.<sup>222</sup> Proportional hazards assumption was tested for all potential confounders that were included in the final model and all variables conformed to the assumption of proportionality. Possible confounding or effect modification by the following known risk factors were considered in the analyses: age (categorized into three groups: <60, 60-70, or >70 years), sex, education ( $\leq 9$ , 10-12, or  $\geq 13$  years), BMI (<25, 25-29.9, or  $\geq 30$  kg/m<sup>2</sup>), tobacco smoking status (never smoking, previous smoking, or current smoking of any tobacco), alcohol drinking (quartile of total alcohol intake per week), energy intake (quartile of total energy intake per day), gastroesophageal reflux symptoms (yes or no), gastric ulcer (yes or no), duodenal ulcer (yes or no), and diabetes (yes or no). Sex-stratified analyses were also performed, but we displayed only the results for men and women combined, and for men separately since the number of female cases was too few to analyze separately.

All *P* values presented are two-sided and *P* < 0.05 was considered statistically significant.

### 5.1 PAPER I

#### **Study participants**

In total, 618 case patients were recruited; including 189 esophageal adenocarcinoma cases (participation rate 88%), 262 gastroesophageal junctional adenocarcinoma cases (84%), and 167 esophageal squamous cell carcinoma cases (73%). The number of control subjects was 820 (73%). The participants with more than 10% missing values of food items were excluded, leaving 181 cases of esophageal adenocarcinoma, 255 cases of gastroesophageal junctional adenocarcinoma, 158 cases of esophageal squamous cell carcinoma, and 806 controls for final analyses (**Table 4**). The average intakes of lignans were 1754.6 µg/day among controls and 1645.4 µg/day among cases.

#### **Lignans and esophageal or gastroesophageal junctional adenocarcinoma**

Dose-dependent associations were observed between lignan intake and esophageal adenocarcinoma, as well for gastroesophageal junctional adenocarcinoma (all *p* for trends <0.05) (**Paper 1, Table 2**). The adjusted ORs indicated a 35% and a 63% reduced risk in the highest lignans exposure quartile compared to the lowest quartile, for esophageal adenocarcinoma and gastroesophageal junctional adenocarcinoma, respectively. Similar results were found in analyses of men only (**Paper 1, Table 3**). The number of female cases was too low to allow statistically robust estimates, but the point ORs were also decreased in women consuming higher levels of lignans. When combining the cases of esophageal and gastroesophageal junctional adenocarcinoma in the analysis, a stronger dose-dependent decreased risk was found with higher intake of lignans (**Figure 8**).

#### **Lignans and esophageal squamous-cell carcinoma**

After adjustment for confounding factors, no clear trend remained in the association between dietary lignans and risk of esophageal squamous cell carcinoma (*p* for trend=0.25), although a decreased point OR was indicated in the highest exposed quartile compared to the lowest (OR 0.66, 95% CI 0.38-1.15) (**Paper 1, Table 2; Figure 8**).

**Conclusion:** This study indicates that dietary intake of lignans is associated with a decreased risk of adenocarcinoma of the esophagus and gastroesophageal junction, while no clear association was found for esophageal squamous-cell carcinoma.

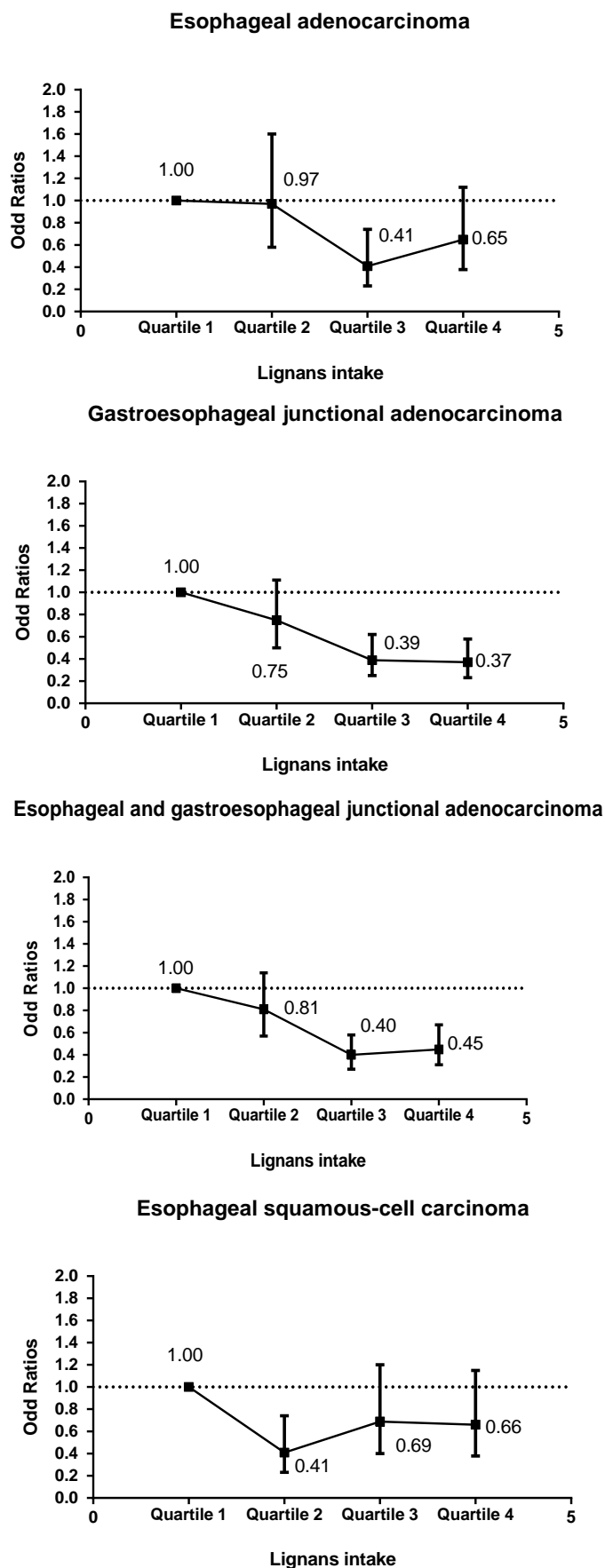
**Table 4.** Baseline characteristics of esophageal cancer case and control participants in a Swedish population-based case- control study.

<b>Characteristic</b>	<b>Control Number (%)</b>	<b>Esophageal adenocarcinoma Number (%)</b>	<b>Gastroesophageal junctional adenocarcinoma Number (%)</b>	<b>Esophageal squamous- cell carcinoma Number (%)</b>
<b>Gender</b>				
Male	667 (82.8)	158 (87.3)	216 (84.7)	114 (72.1)
Female	139 (17.2)	23 (12.7)	39 (15.3)	44 (27.9)
<b>Age (years)</b>				
<55	127 (15.8)	21 (11.5)	52 (20.4)	13 (8.3)
55-64	185 (23.0)	38 (20.1)	57 (22.4)	58 (36.7)
65-74	317 (39.2)	88 (48.6)	105 (41.2)	62 (29.2)
≥75	177 (22.0)	34 (18.8)	41 (16.0)	25 (15.8)
<b>Education (years)</b>				
≤9	489 (60.7)	136 (75.1)	166 (65.1)	114 (72.2)
10-12	158 (19.6)	22 (12.2)	54 (21.2)	23 (14.6)
≥13	159 (19.7)	23 (12.7)	35 (13.7)	21 (13.2)
<b>Body mass index (kg/m<sup>2</sup>)</b>				
<18.5	9 (1.1)	1 (0.6)	3 (1.2)	5 (3.1)
18.5-24.9	553 (69.0)	75 (41.4)	139 (54.5)	102 (64.6)
25.0-29.9	215 (26.8)	84 (46.4)	89 (34.9)	41 (26.0)
≥30.0	25 (3.1)	21 (11.6)	24 (9.4)	10 (6.3)
<b>Tobacco smoking</b>				
Never	321 (39.8)	55 (30.4)	42 (16.5)	20 (12.6)
Ever	309 (38.4)	84 (46.4)	121 (47.5)	42 (26.6)
Current	176 (21.8)	42 (23.2)	92 (36.0)	96 (60.8)
<b>Alcohol consumption (g/week)</b>				
0	128 (15.9)	35 (19.3)	31 (12.2)	15 (9.5)
0-15	214 (26.5)	53 (29.2)	72 (28.2)	32 (20.3)
16-70	286 (35.5)	51 (28.2)	77 (30.2)	38 (24.0)
>70	178 (22.1)	42 (23.2)	75 (29.4)	73 (46.2)
<b>Physical activity<sup>a</sup></b>				
I -low	179 (22.2)	45 (24.9)	53 (20.8)	36 (22.8)
II	256 (31.8)	53 (29.3)	71 (27.8)	55 (34.8)
III	217 (27.0)	42 (23.1)	68 (26.7)	36 (22.8)
IV-high	154 (19.0)	41 (22.7)	63 (24.7)	31 (19.6)
<b>Reflux</b>				
Yes	131 (16.3)	110 (60.8)	74 (29.0)	25 (15.8)
No	675 (83.7)	71 (39.2)	181 (71.0)	133 (84.2)
<b>Dietary phytoestrogen intake (µg/day)</b>				
Total lignans <sup>b</sup>	1754.6	1709.3	1619.2	1607.7

<sup>a</sup> Physical activity was measured by a combination of 12 variables, reflecting activities during leisure time and at work.

<sup>b</sup> Including secoisolariciresinol, matairesinol, lariciresinol, isolariciresinol, pinoresinol, syringaresinol, and medioresinol.

**Figure 8.** Multivariable-adjusted odds ratios and 95% confidence intervals for esophageal cancer in relation to lignan intake in a Swedish nationwide case-control study.

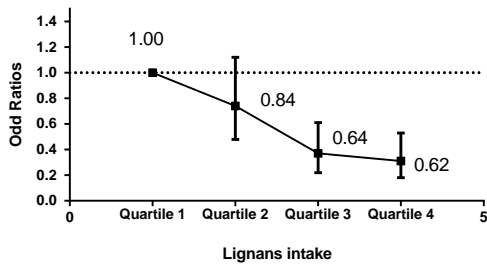


By sex:

**Women**

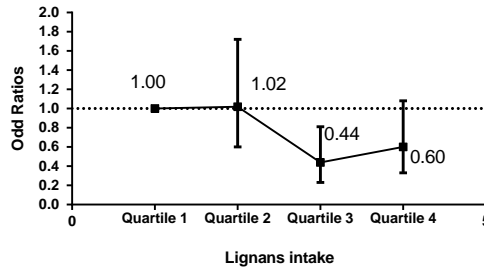
Not available

**Gastroesophageal junction adenocarcinoma**

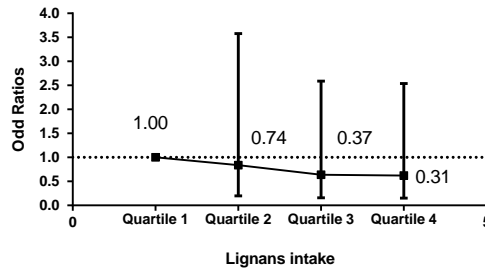


**Men**

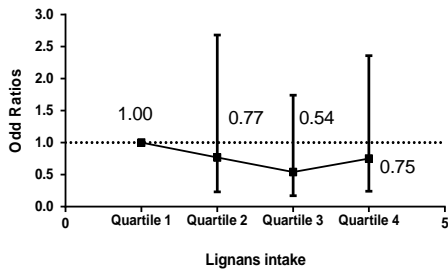
**Esophageal adenocarcinoma**



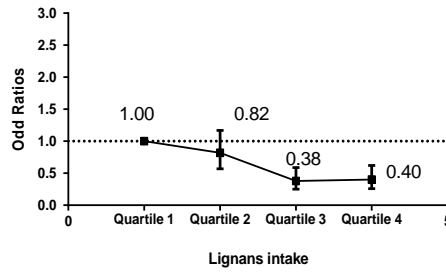
**Gastroesophageal junction adenocarcinoma**



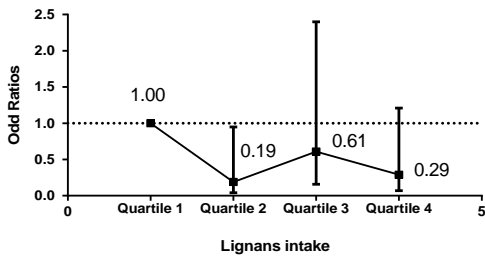
**Esophageal and gastroesophageal junction adenocarcinoma**



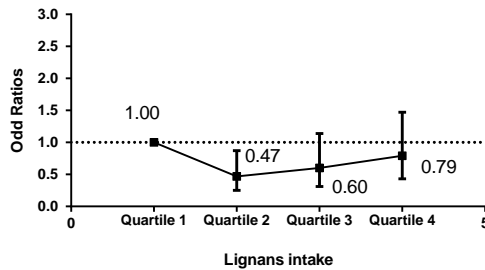
**Esophageal and gastroesophageal junction adenocarcinoma**



**Esophageal squamous-cell carcinoma**



**Esophageal squamous-cell carcinoma**



## 5.2 PAPER II

### *Dietary intake of lignans and serum enterolactone*

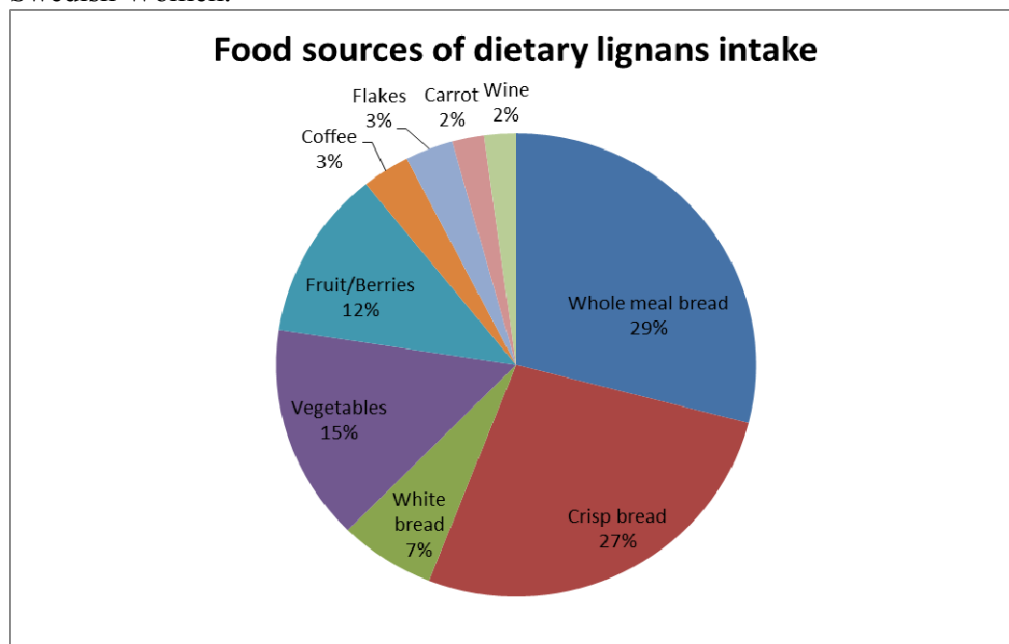
Among the 135 study participants aged 55-75 years, the average energy-adjusted dietary intake of total lignans was 1,616 µg/day (standard deviation ±424 µg/day) according to the FFQ-87, and 1,516 µg/day (±409 µg/day) for the FFQ-97 (**Paper II, Table 1**). The estimates of dietary lignan intake were different for the two FFQs ( $t=3.2$ ,  $p=0.002$ ). The mean concentration of serum ENL was 23.2 nmol/L (±15.4 nmol/L) (**Paper II, Table 2**). Approximately 60% of the daily intake of total lignans was derived from bread, while fruit and vegetable intake accounted for 25% (**Figure 9**).

### *Correlation between dietary lignans and serum enterolactone*

The Pearson's correlation coefficients between lignan intake and serum concentration of ENL are presented in **Paper II, Table 1**. Dietary lignans assessed by the FFQ-97 were statistically significantly correlated with serum ENL in the crude and multivariably adjusted models ( $r=0.16$ ,  $p=0.06$ ;  $r=0.22$ ,  $p=0.01$ , respectively), as well as when converted lignan values were used ( $r=0.17$ ,  $p=0.04$ ;  $r=0.24$ ,  $p=0.006$ , respectively) (**Table 5**). However, no such correlation was found for the FFQ-87 with lignan intake ( $r=0.09$ ,  $p=0.30$ ), nor with converted lignans ( $r=0.12$ ,  $p=0.19$ ). The correlation between the two FFQs was statistically significant ( $r=0.59$ ,  $p<0.0001$ ). Pearson's correlations between intake of total lignans and of converted lignans and serum ENL in BMI subgroups are shown in **Table 6**. The highest correlation was observed in obese women ( $BMI \geq 30.0 \text{ kg/m}^2$ ).

**Conclusion:** The present study shows that the validity of FFQ-based estimates of dietary lignan intake depends on the number of food items containing lignans in the FFQ, and potentially the BMI of the study subjects.

**Figure 9.** Food sources for major contribution of dietary intake of lignans among 135 Swedish Women.



**Table 5.** Pearson’s correlation between energy-adjust dietary intake of lignans based on the food frequency questionnaire (FFQ) and serum concentration for enterolactone.<sup>a</sup>

Intake	Serum vs. FFQ-87 <sup>b</sup>			Serum vs. FFQ-97 <sup>b</sup>		FFQ-87 vs. FFQ-97
	No.	Crude (P-value)	Adjusted <sup>c</sup> (P-value)	Crude (P-value)	Adjusted <sup>c</sup> (P-value)	Crude (P-value)
Total lignans	135 <sup>d</sup>	0.06 (0.47)	0.09 (0.30)	0.16 (0.06)	0.22 (0.01)	0.59 (<0.0001)
Converted lignans <sup>e</sup>	135 <sup>d</sup>	0.08 (0.33)	0.12 (0.19)	0.17 (0.04)	0.24 (0.006)	0.62 (<0.0001)

<sup>a</sup> All dietary intakes of lignans and serum enterolactone were log-transformed.

<sup>b</sup> FFQ-87: food frequency questionnaire, 1987 version (45 food items containing lignans); FFQ-97: food frequency questionnaire, 1997 version (65 food items containing lignans).

<sup>c</sup> Adjusted for age, body mass index (BMI <25, 25-29.9, ≥30 kg/m<sup>2</sup>), constipation (yes/no), gastrointestinal disease history (yes/no), and diabetes (yes/no).

<sup>d</sup> Five outliers (95% CI) were excluded.

<sup>e</sup> Expected amount of dietary lignans was converted to enterolactone in the intestine using conversion factors: matairesinol=0.62, secoisolariciresinol=0.72, lariciresinol=1.01, pinoresinol=0.55, syringaresinol=0.44, pinoresinol=0.55, syringaresinol=0.04, medioresinol=0.8.

**Table 6.** Pearson correlation between energy-adjusted dietary intake of lignans based on the food frequency questionnaire (FFQ) and serum concentration of enterolactone stratified by body mass index.<sup>a</sup>

BMI (kg/m <sup>2</sup> ) <sup>e</sup>	No.	Serum vs. FFQ-87 <sup>b</sup>		Serum vs. FFQ-87_converted <sup>c</sup>		Serum vs. FFQ-97 <sup>b</sup>		Serum vs. FFQ-97_Converted <sup>c</sup>	
		Crude (P-value)	Adjusted <sup>d</sup> (P-value)	Crude (P-value)	Adjusted <sup>d</sup> (P-value)	Crude (P-value)	Adjusted <sup>d</sup> (P-value)	Crude (P-value)	Adjusted <sup>d</sup> (P-value)
1 (<25)	54	0.007 (0.96)	-0.005(0.97)	0.04 (0.76)	0.03 (0.85)	0.13 (0.35)	0.14 (0.34)	0.13 (0.35)	0.14 (0.33)
2 (25.0-29.9)	50	0.11 (0.47)	0.11 (0.48)	0.11 (0.44)	0.12 (0.43)	0.08 (0.57)	0.14 (0.36)	0.08 (0.60)	0.19 (0.23)
3 (≥30.0)	29	0.13 (0.50)	0.24 (0.26)	0.15 (0.45)	0.27 (0.20)	0.36 (0.06)	0.38 (0.07)	0.37 (0.05)	0.38 (0.07)
<i>P-value for trend<sup>f</sup></i>		0.15	0.12	0.15	0.13	0.09	0.04	0.12	0.05

<sup>a</sup> All dietary intakes of lignans and serum enterolactone were log-transformed.

<sup>b</sup> FFQ-87: food frequency questionnaire, 1987 version (45 food items containing lignans); FFQ-97: food frequency questionnaire, 1997 version (65 food items containing lignans).

<sup>c</sup> Expected amount of dietary lignans could be converted to enterolactone in the intestine.

<sup>d</sup> Adjusted for age, constipation (yes/no), gastrointestinal disease history (yes/no), diabetes (yes/no), when applicable.

<sup>e</sup> Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters; two subjects had missing BMI values.

<sup>f</sup> Test for trend across BMI categories was conducted by assigning the median of BMI in each BMI category and then treating these values as a continuous variable



### 5.3 PAPER III

#### **Study participants**

The study included 81,670 study participants, including 36,697 women (44.9 %) and 44,973 men (55.1 %). Sex-specific baseline characteristics of these participants divided by quartiles of dietary lignan intake in energy density are shown in **Paper III, Table 1**. Compared with women and men with low intake of lignans, those with higher intake were more likely to have a postsecondary education, and have history of diabetes and ulcer, and less likely to smoke, drink alcohol and have reflux, while the BMI was similar between groups. During an average of 9.9 years of follow-up, 83 cases (69 male and 14 female) of esophageal or gastroesophageal junctional adenocarcinoma and 128 cases (72 male and 56 female) of gastric adenocarcinoma were identified.

#### **Lignan intake and esophageal and gastric adenocarcinoma**

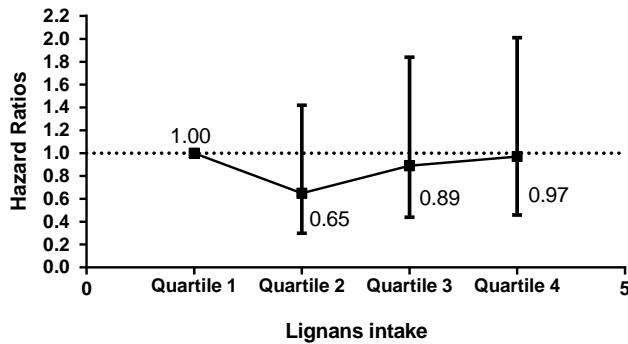
The HRs between higher dietary lignan intake and risk of esophageal or gastric adenocarcinoma indicated slight inverse point risk estimates, but no statistically significantly decreased risks were identified, and no dose-response trends were observed (**Figure 10**). In the entire cohort (men and women combined), the adjusted HR of adenocarcinoma of esophagus or gastroesophageal junction in the highest quartile of lignan intake was 0.97 (95% CI: 0.46-2.01) (**Paper III, Table 2**). The corresponding HR for gastric adenocarcinoma was 0.87 (95% CI: 0.51-1.52) (**Paper III, Table 2**). Similar results were observed when only men were analyzed.

**Conclusion:** This study found no clear decreased risk of esophageal or gastric adenocarcinoma among persons consumed higher amounts of lignans. A reason for the non-significant association in the present cohort study might be the limited number of cases.

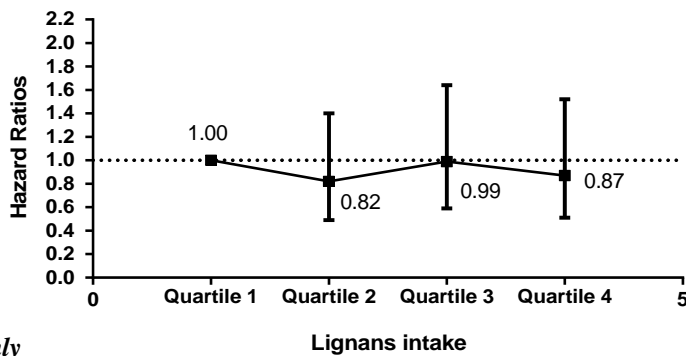
**Figure 10.** Multivariable-adjusted hazard ratios and 95% confidence interval of esophageal and gastric adenocarcinoma in relation to lignan intake.

Total cohort

**Esophageal and gastroesophageal junctional adenocarcinoma**

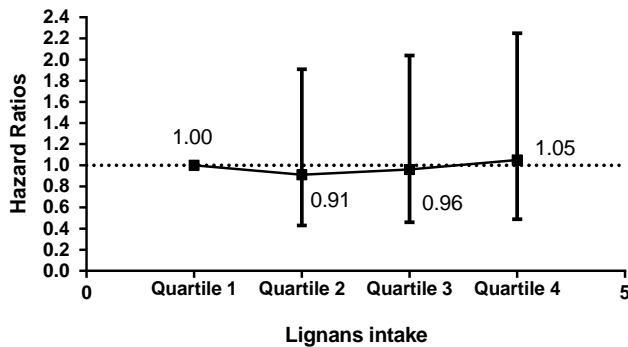


**Gastric adenocarcinoma**

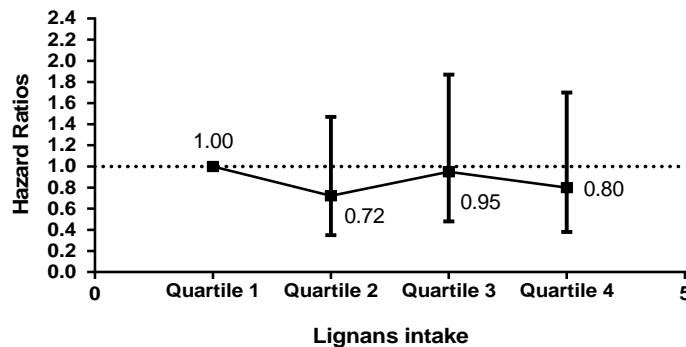


Men only

**Esophageal and gastroesophageal junctional adenocarcinoma**



**Gastric adenocarcinoma**



## 5.4 PAPER IV

### Study participants

The study included 594 cases of esophageal cancer and 806 controls. Controls had a higher intake of tea, lettuce, whole grain bread and mixed vegetables than the case groups, but they consumed less milk (t -test,  $p < 0.05$ ). No differences were found for intake of tomatoes and wine. Intake of quercetin and lignans were higher among controls than in each case group (t -test,  $p < 0.05$ , data not shown), while intake of resveratrol was similar for controls and cases (t-test,  $p = 0.68$ ). The simplified pattern scores were positive in the control group, but negative in all three case groups.

### Food Groups Contributing to the Lignans, Quercetin and Resveratrol Pattern Score

Tea, wine, lettuce, mixed vegetables, tomatoes, milk, and whole grain bread, with a factor loading  $\geq |0.2|$ , were the most important food items defining level of dietary lignans, quercetin, and resveratrol, based on the estimation of the RRR procedure, and retained in the simplified food pattern (**Table 7**). Pearson's correlation coefficients of the food pattern scores with food items were slightly lower for the simplified food pattern score compared with the original food pattern. Both the original and simplified food pattern scores correlated strongest with tea and wine intake, which explained most of the variation in the original food pattern scores. Milk intake was the only item negatively associated with the studied pattern scores (**Table 7**).

### Dietary Pattern Characterized by Lignans, Quercetin and Resveratrol and Risk of Esophageal Cancer by Histologic type

The identified food pattern characterized by lignans, quercetin and resveratrol was strongly associated with risk of esophageal adenocarcinoma, gastroesophageal junctional adenocarcinoma, and esophageal squamous-cell carcinoma (**Figure 11**). Dose-dependent associations were found between higher simplified food pattern scores and a decreased risk of each of the studied tumors (all  $p$  for trend  $< 0.05$ ). Comparing the extreme quintiles (quintile 5 versus quintile 1) of scores in the fully adjusted model showed decreased risk estimates of esophageal adenocarcinoma (OR 0.24, 95% CI: 0.12-0.49), esophageal squamous-cell carcinoma (OR 0.31, 95% CI: 0.15-0.65), and gastroesophageal junctional adenocarcinoma (OR 0.49, 95% CI: 0.28-0.84) (**Paper IV, Table 3**).

**Conclusion:** A dietary pattern characterized by high combined intake of lignans, quercetin, and resveratrol was strongly associated with a decreased risk of all types of studied esophageal cancer in this study. High food pattern scores for these three phytochemicals were derived mainly from a high consumption of tea, wine, lettuce, mixed vegetables, tomatoes, and whole grain bread, and a low consumption of milk.

**Table 7.** Food groups mainly contributing to a high phytochemicals (lignans, quercetin, and resveratrol) food pattern score.

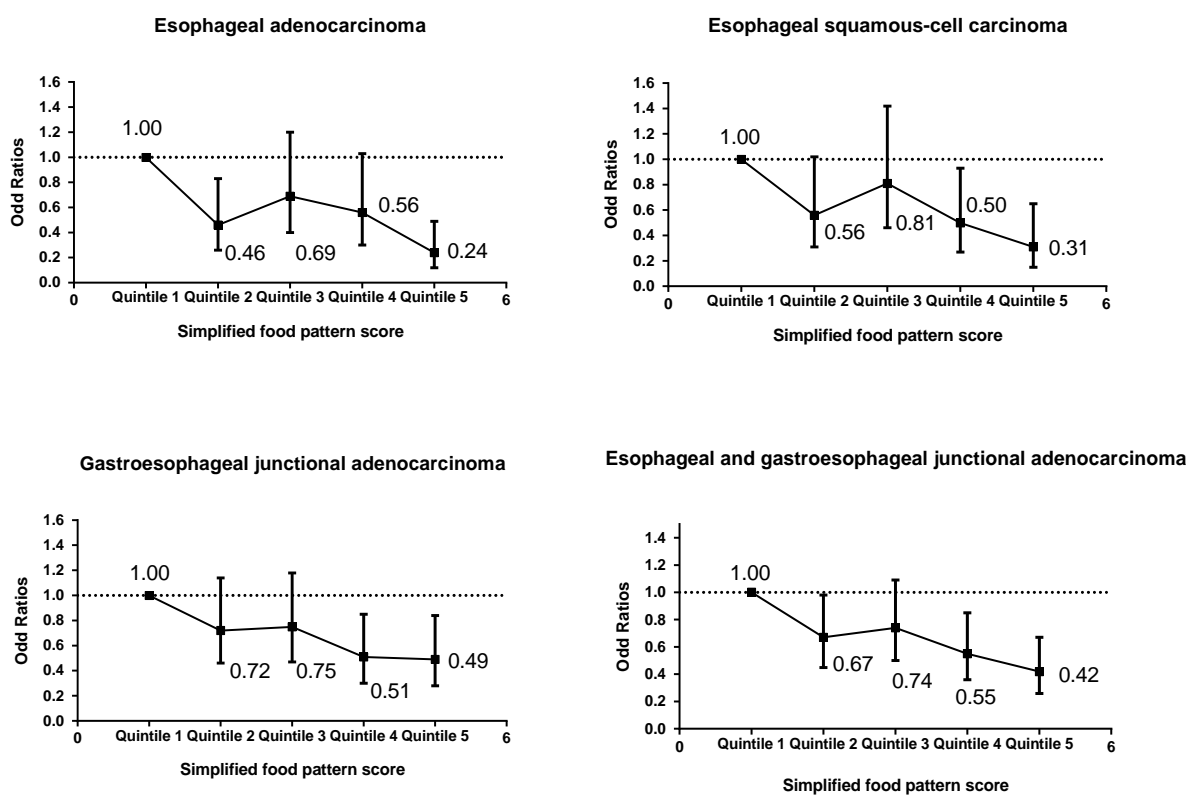
<i>Dietary Intake<sup>a</sup></i>	<b>Original food pattern score<sup>b</sup></b>		<b>Simplified food pattern score<sup>c</sup></b>
	Pearson's correlation coefficient	Factor loading	Pearson's correlation coefficient
Tea	0.70	0.48	0.70
Wine	0.52	0.36	0.71
Lettuce	0.39	0.27	0.37
Mixed vegetables	0.34	0.23	0.28
Tomatoes	0.32	0.22	0.29
Milk	-0.31	-0.21	-0.28
Whole grain bread	0.29	0.20	0.10

a. The food intake was expressed in energy-adjust densities as per 1000 kcal/day.

b. Original food pattern score was estimated by using reduced rank regression analysis. Original food pattern score was estimated by using reduced rank regression analysis.

c. A simplified food pattern score was obtained by summing standardized consumption of tea, wine, lettuce, mixed vegetables, tomatoes, milk and whole grain bread intake in energy-adjust densities.

**Figure 11.** Multivariable-adjusted odds ratios and 95% confidence intervals for esophageal cancer in relation to dietary pattern characterized by intakes of lignans, quercetin, and resveratrol.



### 6.1 METHODOLOGICAL CONSIDERATION

#### 6.1.1 Study design

##### *Population-based case-control study*

Case-control studies compare persons with the disease (case) with persons without that specific disease (control) (**Figure 12**). The proportions of exposure of interest are then compared between case and control groups. Compared to a prospective cohort study, the case-control design is relatively inexpensive and requires fewer subjects. This design is therefore particularly valuable for studying a rare disease with a long expected latency period between the exposure and the disease. This design also has advantages for studying associations of a disease with several exposures, which is particularly valuable for detecting unknown risk factors for a rare disease, such as esophageal cancer. However, a case-control study usually collects data retrospectively, when the disease status is already known. This strategy is potentially vulnerable to information bias, i.e. recall bias, which might differ between cases and controls. Another major pitfall of case-control design is selection bias. A main source of selection bias is the inappropriate selection of controls. The selected controls should be similar to the cases in all respects other than having the disease in question, or be representative of all persons without the disease in the population from which the cases are selected. This bias could be largely reduced by using a nationwide population-based design, which was used in **Paper I** and **IV**. We identified the cases and controls throughout the whole Swedish population. This so called “population-based” design reduces the selection bias by selecting subjects representing the situation of the whole Swedish population.

##### *Validation study*

The FFQ is the most used measurement method to assess dietary nutrient or food intake. The interpretation of epidemiologic data based on the FFQ depends on the validity of this dietary measurement. *Validity* refers to the degree to which the questionnaire actually measures the aspect of diet that it was designed to measure, and it is usually tested by comparing the data based on the questionnaire with those measured by a more accurate method, that is, a “*gold standard*”. Virtually, no truly perfect test that can be used as a gold standard exists, since all measurements have errors, although these differ in their magnitude. In absence of a perfect test, the best available comparison method is usually considered as a gold standard, i.e. the application of a biomarker for a specific nutrient intake. A crucial point in the selection of a gold standard is that its errors should be independent of the method being evaluated. Therefore, the fundamental advantage of using a biomarker is that its measurement errors are uncorrelated with errors in the FFQ.

After consumption, plant lignans are converted by the gut microflora into mammalian enterolignans, primarily ENL. ENL is widely used as the gold standard biomarker to calibrate dietary lignan intake. However, biomarkers have been criticized as an

imprecise measurement of diet, since they can be influenced by many factors, such as differences in absorption and metabolism, day-to-day variation, and laboratory measurement errors. In **Paper II**, only one single sample was analyzed for serum concentration of ENL. Since the biological level is dynamic, fluctuating substantially over time, one measurement at a time point may not reflect long-term intake, which the questionnaire was designed to evaluate. These errors in total result in weakening any association between the biomarker and the questionnaire, even when the dietary measurement is precise and accurate. In order to overcome the drawback of using a biomarker, we employed several strategies to reduce the extraneous variation in the biomarker. Several factors that can influence the metabolism of plant lignans and the absorption of mammalian lignans were added into the adjustment, i.e. age, constipation, diabetes, and gastrointestinal disease history. Since BMI is indicated as an important determinant of the serum ENL concentration, additional analyses stratified by BMI subgroups were also conducted. Further criteria should be considered to eliminate the random laboratory errors, i.e. by obtaining biological samples at several time points with a quantity of samples per subject and having larger sample size.

### ***Cohort study***

In a cohort study, the investigator selects a group of exposed individuals (free of disease) and a group of non-exposed individuals (free of disease) and follows up both groups to compare the occurrence of the outcome under study (**Figure 12**). It is particularly valuable for assessing the effects of rare exposures, and to allow the investigation of multiple exposures. Another advantage of a cohort study is that the prospective design minimizes the potential for recall and other biases in assessing the exposure and has validity of the exposure assessment. Cohort studies often involve follow-up of populations over a long period in order to determine whether the participants develop the disease of interest. Therefore, such investigations are usually time and economy-consuming. Furthermore, loss to follow-up is a major source of bias in most cohort studies. If the loss to follow-up is related to both exposure and outcome, the incidence rates calculated in the exposed and non-exposed groups will clearly be difficult to interpret. Likewise, non-participation and non-response can also introduce biases that can complicate the interpretation of the study findings.

In **Paper III**, the data was prospectively collected from the SMC and the COSM. In Sweden, the whole population can be linked to various national registers by using the unique personal identity number. The nationwide complete registers, i.e. Cancer Register, Patient Register, National Diabetes Register, Emigration Register, Register of Total Population, and the Death Register provide valid follow-up of all cohort members.



symptoms, use of tobacco, alcohol, and all studied covariates. This similarity indicates that non-participation among controls did not introduce an important selection bias.

Selection bias is less of a problem in a prospective cohort study, since the exposed and unexposed subjects are free of disease at the entry of follow-up. However, it ought to be kept in mind that a selection bias could be introduced in a prospective design when the loss of follow-up is different among exposed and non-exposed groups. The linkage to various nationwide registers in **Paper III** actually provides virtually complete follow-up for all participants, and therefore minimizes selection bias.

### ***Information bias***

#### 1) Misclassification of exposure

Some degree of misclassification of lignan intake due to measurement error associated with the FFQ was unavoidable. For example, the FFQ might have overestimated a number of food groups, particularly lignan intakes from fruit and vegetables,<sup>213</sup> and the FFQ was not designed specifically for lignan intake, making it difficult to obtain full coverage of related food sources. In a prospective study (**Paper III**), the misclassification of lignan measurement tends to be non-differential, that is, similar in cases and controls. It might lead to attenuation of possible effects, and this may explain the lack of statistically significant associations in that study. Despite this misclassification and other unavoidable errors by using the FFQ in a prospective study, the validation study (**Paper II**) indicated that the FFQ was a reasonably useful tool to assess dietary intake of lignans.<sup>223</sup> On the other hand in the studies with retrospective case-control design (**Paper I and IV**), the systematic error is more likely to be differential, whereby the rate of misclassification differs in case and control groups. Differential misclassification can result in associations that prove to be wrong. In **Paper I and IV**, the non-blinded professional interviewers conducted the interviews, which might introduce information bias differentially. However, all interviewers were unaware of the study hypothesis and trained to treat cases and controls in a strictly equal way. Recall bias is a common type of differential misclassification that often occurs in case-control studies. It is widely recognized that cases might be more concerned in recalling their dietary habit and therefore result in more over-reporting of true exposure than controls, who may be less likely to recall a true exposure. The net effect of the bias is to exaggerate the difference between cases and controls and lead to a higher likelihood of detecting statistically significant associations.

In **Paper I and IV**, the lignan exposure was assessed based on diet 20 years prior to the interview, resulting in increased risk of misclassification of the true exposure, but we chose this approach to assess dietary habits during a time window when the interaction between diet and cancer is possible. This method of assessment of dietary items 20 years earlier has been validated with good results and showed that such assessment captures a combination of previous and current dietary habits.<sup>196</sup>

#### 2) Misclassification of outcome

In **Paper I and IV**, a comprehensive organization for the rapid ascertainment of cases was used. In order to reduce misclassification of the tumor site or histologic type,



uniform routines for the documentation of tumors of the patients were introduced in all participating departments.

In **Paper III**, all incident cases of esophageal and gastric adenocarcinoma were identified through the Swedish National Cancer Register, which was established to be 63% complete for esophageal adenocarcinoma and 98% complete for gastric adenocarcinoma.<sup>200,201</sup> Specifically, the validity of using the Swedish National Cancer Register to identify esophageal and gastric cancer was excellent, 73% for esophageal and gastric adenocarcinoma, and 93% for esophageal squamous cell carcinoma. Therefore, the large and validated national register minimized the possibility of underascertainment of cancer cases. Furthermore, in order to avoid detection bias, we excluded all participants diagnosed with any cancer during the first year of follow-up.

### ***Confounding***

Confounding, which might explain the observed association (or null-association) is due to the effect of a third factor that is both a risk factor for the disease and is associated with the exposure in question. Failure to account for confounding can result in spurious associations. Three criteria must be fulfilled in order to refer to a factor as a confounder. First, the factor needs to be associated with the study exposure. Second, the factor needs to be associated with risk of the disease of interest. Third, the factor should not be the effect of exposure, namely an intermediate step in the causal pathway between the exposure and outcome. Confounding is a most important threat to the validity of data from observational studies in general. In nutritional epidemiologic studies, it is extremely difficult to exclude confounding since intake of various food items and nutrients are often strongly correlated.

For example, the main food sources of lignans in the Swedish population are whole grain bread, soft bread, and crisp bread, which are also the major sources for cereal fiber intake. Therefore, lignan intake is highly correlated with cereal fiber intake. Lignans might be a kind of derivative component of cereal fiber and therefore might act as an intermediate step in the casual pathway between cereal fiber intake and esophageal cancer. Hence, cereal fiber intake was not considered as a confounder for the potential association between lignan intake and esophageal cancer in the current thesis.

In **Paper I** and **IV**, the controls were selected by age and sex matching in order to mimic the distribution among cases. This strategy, together with the adjustment for all relevant potential confounders for esophageal cancer in the analysis, would minimize the confounding effect of these factors. In **Paper III**, information was missing on some important potential confounding factors, including *Helicobacter pylori* infection, the main risk factor of gastric adenocarcinoma, and use of antibiotics, which might reduce serum concentrations of ENL.

### **Random error (precision)**

One of the advantages in **Paper I** and **IV** is the relatively large sample size, reducing the risk of random error. We included virtually all cases of esophageal and gastroesophageal junctional adenocarcinoma in Sweden during a 3-year period.

Limitations of **Paper II** include the small sample size, although it was not smaller than that of other phytoestrogen validation studies. Another limitation that could increase the risk of random error was that only a single fasting blood sample was used as a gold standard. The high intra-individual variation with poor precision when using a single measurement might lead to misclassification of the ENL exposure. However, the concentration of serum ENL in the study was in line with the concentrations observed in other Swedish studies. In **Paper III**, although the size of total cohort is large, we obtained only a limited number of esophageal and gastric adenocarcinoma cases, suggesting limited power.

## 6.2 INTERPRETATION AND IMPLICATION

### **Lignan intake and risk of esophageal cancer**

Results from **Paper I** indicate that dietary intake of lignans is associated with a decreased risk of adenocarcinoma of the esophagus and gastroesophageal junction, but not for esophageal squamous cell carcinoma. The beneficial effects of lignans on esophageal and gastroesophageal junctional adenocarcinoma suggested in this study might be attributed to their structural similarities to estrogen. The estrogenic effects of lignans are widely assumed to be mediated by the mammalian metabolite ENL and via activating estrogen receptors (ERs).<sup>164</sup> Evidence from previous studies suggest that the protective effect of estrogen on esophageal cancer is mediated by the ERs.<sup>165</sup> Since the presence of ERs has been identified in esophageal tissue,<sup>166,224</sup> a linkage between mammalian lignans and esophageal cancer via ERs is possible. Isoflavone is another type of phytoestrogen that has been found to have an anti-carcinogenic effect. However, isoflavones are commonly exhibited in soybeans, which are rare in the Western diet. Therefore, we did not include isoflavones in the current study. It is important to state that the results identified here are specifically related to a diet rich in phytoestrogen lignans, not supplementation with the same compounds and the results might be related to other compounds in the same diet acting on their own or in a synergistic manner with phytoestrogen lignans.

Although the above case-control study suggested a beneficial effect of lignan intake in the development of esophageal cancer, the results from our prospective cohort study **Paper III** found no clear support for this hypothesis. We found inverse point risk estimates of esophageal and gastroesophageal junctional adenocarcinoma among those with higher dietary lignan intake compared to the lowest category, but they were not statistically significant. Although this cohort study did not provide strong evidence for any inverse association, the results from these two studies were to some extent consistent. A reason for the non-significant results in the present cohort study might be ascribed to the limited number of cases. Furthermore, since the misclassification of the exposure in a prospective cohort study design tends to be non-differential, i.e. similar in cases and non-cases, it might lead to attenuation of possible effects, and this may also contribute to the lack of statistically significant associations in the present study. Further prospective studies with larger sample size are warranted before we can establish the role of lignans in the development of esophageal and gastric adenocarcinoma.

### **Validity of FFQ-based assessment for lignan intake**

The results from **Paper II** show that the validity of FFQ-based estimates of dietary lignan intake depends on the number of food items containing lignans in the FFQ, and potentially the BMI of the study subjects.

The reasons for the low correlation between lignan intake and serum ENL might be diverse. FFQs might overestimate the consumption of a number of food groups, particularly lignan intake from fruit and vegetables,<sup>213</sup> and the FFQs were not designed specifically for lignan intake; thus it was difficult to obtain full coverage of related food sources. On the other hand, concentration of ENL might vary substantially over the course of a single day, or different seasons. Furthermore, potentially large inter-individual variation in the metabolism of plant lignans into mammalian ENL cannot be ignored.<sup>225</sup> Upon ingestion, plant lignans are transformed into their immediate precursor END and then ENL by certain gut microflora.<sup>121,226</sup> Therefore, lack of certain gut microflora can result in different END-to-ENL ratios. Moreover, persons regularly consuming certain lignan-rich foods, such as fruit, vegetables and fiber-rich foods have more efficient transformation of plant lignans into mammalian ENL.<sup>227,228</sup> Smoking and high BMI might decrease the serum concentration of ENL, while constipation might enhance the production of ENL due to the decrease of intestinal motility.<sup>132,229</sup>

To improve the assessment of phytoestrogens using FFQs some modifications might be warranted. For example, some improvements might be needed to develop a lignan-specific questionnaire, in which important exposures such as age, education, and BMI will also be included. Furthermore, several criteria should be considered when adopting a biomarker to calibrate dietary assessment, e.g. by obtaining serum samples at several time points and calculating mean values rather than single values. Although hampered by low statistical power, it was interesting that obese participants seemed to show a higher correlation between the estimate of plant lignan intake and serum concentration of ENL. Speculatively, obese people might be more prone to recalling healthy dietary habits, such as intake of fruit and vegetables, compared to non-obese people. The difference in assessment of dietary lignan intake using the FFQ-97 and the FFQ-87 might be due to the fact that the FFQ-97 (65 food items containing lignans) evaluated more lignan-containing food items than the FFQ-87 (45 food items containing lignans), and the fact that some questions in the FFQ-97 were more precise than those in the FFQ-87. Participants were asked to report food intake frequency within eight categories ranging from 'never/ seldom' to '4 times/day' in the FFQ-87, whereas they were asked about the precise frequency (open answers, not pre-specified categories) for commonly consumed food items, e.g. tea, in the FFQ-97. In fact, there were differences between the FFQ-87 and the FFQ-97 in terms of assessment of tea.

In summary, this validation study indicates that the correlation between plant lignan intake based on assessment with the FFQ and serum concentration of ENL is limited, which can partly depend on varying metabolism of lignans. Therefore, a cautious interpretation of FFQ-based results regarding lignan exposure is recommended, and direct measurements of serum ENL might be preferred.

## **Dietary patterns and esophageal cancer**

**Paper IV** demonstrates that a dietary pattern characterized by high intake of lignans, quercetin and resveratrol was inversely associated with risk of esophageal cancer, including gastroesophageal junctional adenocarcinoma. With increasing food pattern scores, intake of tea, wine, lettuce, mixed vegetables, tomatoes, and whole grain bread increases intake, while intake of milk decreases intake.

The RRR method used in the present study is a powerful tool in describing overall dietary habit and evaluating the potential association with outcome. It integrates the characteristics of both a *priori* and a *posteriori* approaches by defining a group of food variables assumed to be linked to the outcome and explaining variation in interested nutrients. The rationale for using this method is the assumption that variation in intake of these nutrients might affect the risk for disease outcome. However, the major concern of RRR, and other dietary pattern analysis, is that it might be difficult to reproduce the results across different study populations. Because of this, we chose the simplified food pattern approach by including only the food items making the most important contributions to resveratrol, quercetin and lignan intake. Thus, the identified simplified food pattern is easily reproducible in other study populations. This novel method has been proven informative in examining associations between dietary patterns and several disease outcomes.<sup>230,231</sup> Nevertheless, it has been a concern that simplification might result in loss of relevant information, therefore is difficult to predict the variance in nutrients. However, in the present study, the Pearson's correlation coefficient between the original and simplified scores was relatively high (0.85), indicating that the loss of information was limited.

In our dietary pattern analysis, tea, with the highest loading factor in the pattern, has been indicated as a rich food resource for intake of quercetin and lignans in several Western populations.<sup>232</sup> Therefore, the fact that tea was a major determinant of the food patterns derived in our study is plausible. It ought to be noted that, during the exposure period of interest (around 1970s), green tea was basically non-existent on the Swedish market. Therefore all tea intake was assumed to be black tea in the current study. Recently, an inverse association between high consumption of black tea and risk of esophageal cancer has also been observed in an Italian case-control study.<sup>233</sup> Evidence from animal research has confirmed the potential anti-carcinogenic effect of black tea, which was observed to reduce tumor growth.<sup>234</sup> Interestingly, this effect was synergistically enhanced by the combination with intake of resveratrol.<sup>235</sup> Wine, which is rich in resveratrol, was another key component in our defined pattern. It is possible that resveratrol contributes to the decreased risk of esophageal cancer among wine drinkers seen in some studies.<sup>43</sup> High intake of fruit and vegetables also contributes to the reduction in esophageal cancer. This finding is consistent with the inverse association we found between intake of fruit and vegetables and risk of esophageal cancer in our earlier study using the same data.<sup>56</sup> This food group is a main source of these phytochemicals. Particularly, tomatoes and lettuce, the important contributors for quercetin intake, were highlighted in our food pattern. Whole grain bread is the most important dietary source of lignan intake in the Swedish population,<sup>223</sup> and other European countries.<sup>236</sup> The highest quartile of whole grain bread intake was associated with reduced risk of esophageal cancer, and it was also one of the components in an identified "healthy" pattern for esophageal adenocarcinoma.<sup>64</sup> The healthy dietary

pattern was also characterized by low intake of milk.<sup>64</sup> A high energy contribution from milk usually associates with low energy intake from fruit and vegetables, which often results in low intake of these three phytochemicals, and high milk consumption might increase the risk of esophageal cancer.<sup>237</sup>

Epidemiologic studies addressing lignans, quercetin, and resveratrol separately in relation to risk of esophageal cancer are scarce. The estrogenic and anti-obesity properties of lignans might contribute to the reduced risk of esophageal cancers found in the present study.<sup>126</sup> Animal research has found that quercetin can induce apoptosis,<sup>238</sup> but no epidemiologic studies have addressed the role of quercetin intake in the development of esophageal cancer. A strong inverse association between quercetin intake and risk of distal gastric cancer has, however, been reported.<sup>239</sup> Resveratrol can exert anti-carcinogenic effects, modulate lipid and lipoprotein metabolism, and have estrogenic properties.<sup>240,241</sup> A food pattern characterized by a high combined intake of these three phytochemicals might exert an anti-carcinogenic effect based on the synergistic effect in suppression of adipogenesis and induction of apoptosis, as indicated in animal research.<sup>191</sup>

In summary, this case-control study indicates that a high dietary intake of lignans, quercetin, and resveratrol (represented by a high intake of tea, wine, tomatoes, lettuce, mixed vegetables, whole grain bread, and a low intake of milk) could prevent the development of esophageal cancer.

### **6.3 FUTURE RESEARCH**

The results of this thesis give rise to questions that will hopefully be addressed in further research.

#### **Future prospective study with a larger sample size**

A protective role of lignan intake in the development of esophageal cancer might exist, but more research is required. Due to the potential estrogenic property of lignans and the fact that obese hormones distribute differently in men and women, the protective effect of lignans has been hypothesized to be female-specific. However, the low female incidence of esophageal cancer in our study did not allow robust estimation of the risk separately in women. Future research should be addressed with a larger sample size of female cases. The lack of association between lignan intake and esophageal cancer detected in the prospective study might be mainly due to the limited statistical power. Much larger, prospective, and well-designed long-term studies are needed to investigate the potential association.

#### **Specific phytoestrogen composition table**

The FFQ is the traditional tool for dietary measurement in nutritional epidemiology. It describes the dietary intake trend in a target population. In most cases, participants are required to recall or record the habitual food intake frequency, portion size, cooking method, and sometimes, the recipe ingredients. The nutrients phytoestrogen are then

calculated by multiplying the food intake frequency, portion size and the nutrients contents based on appropriate food composition tables for the target population. However, a specific food composition table for phytoestrogen is not available for the Swedish population. In the current thesis, the phytoestrogen intake was calculated based on the published phytoestrogen contents data from different countries. These issues together with the existence of numerous factors that could influence the dietary measurement, including varying dietary habit over days/months/seasons/years, reporting biases, lifestyle confounders, etc., mean all the dietary assessments are associated with some extent of measurement error, which might obscure disease-risk associations. Therefore, future efforts should have their emphasis on developing a specific phytoestrogen composition table for the Swedish population.

### **Improvement for phytoestrogen intake assessment**

Various statistical techniques have been developed to reduce dietary measurement errors and to better estimate the usual food intakes. However, given the fact that the measurement error cannot be fully eradicated, true intake is still not really calculated. For these reasons, the dietary biomarker enterolactone measured in biological specimens, such as serum, plasma, urine, are increasingly being used as an alternative instrument for measuring dietary phytoestrogen lignan measurement in modern nutritional epidemiology. The rationale to use biomarkers is that they are objective measurements and are independent of all the biases and errors associated with study subjects and dietary measurement methods. However, the level of biomarker enterolactone is also influenced by various factors, including bioactivity of microflora, interactions between nutrients, genetic backgrounds, gene-gene interactions, laboratory measurement errors, BMI, and medication. These important metabolic and genetic factors may induce the differences in digestion, absorption, transport, metabolism, biotransformation, and excretion of phytoestrogens and finally modulate the correlation of phytoestrogens exposure with risk of esophageal cancer. Currently, very little is known about the possible gene-phytoestrogens or gene-gene interactions and very few studies to date have considered these important factors. It is therefore important for nutritional scientists to use biomarkers for phytoestrogen exposure measurement in future studies, with consideration of a genetic influence. Moreover, given the existence of limitations in both dietary measurements and biomarkers, more advanced statistical methods that combine self-reported nutrient intake with relevant nutrient biomarker can be applied to strengthen the analysis of phytoestrogen-esophageal cancer hypothesis.

## 7 CONCLUSIONS

- High dietary intake of lignans might decrease the risk of adenocarcinoma of the esophagus and gastroesophageal junction.
- The validity of food frequency questionnaire-based estimates of dietary lignan intake is limited and might depend on the number of food items containing lignans in the food frequency questionnaire, and potentially the body mass index of the study subjects. Interpretations made on FFQ-based results regarding lignan exposure should be made with cautiousness.
- A dietary pattern with high intake of lignans, quercetin and resveratrol might counteract the development of esophageal cancer.

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