IDENTIFICATION OF NOVEL MODIFIABLE RISK FACTORS OF GASTRIC ADENOCARCINOMA

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ABSTRACT

Gastric cancer is the fifth most common occurred cancer worldwide. The predominant higher incidence of gastric cancer among males and the diminishing difference after age of 60 years suggests a potential protective effect of female hormones. In **Chapter One**, we found late age at natural menopause was associated with reduced risk of gastric cancer (≥55 vs. ≤ 45years old: HR=0.50, 95% CI: 0.25-0.99). Greater years of menstrual cycling were associated with a decreased risk of gastric adenocarcinoma (4^{th} versus 1^{st} quartile: HR=0.67, 95%CI: 0.46-0.96). Both ever use of oral contraceptives (OCs) and hormone replacement therapy (HRT) were also associated with reduced risk of gastric adenocarcinoma; the HRs (95% CIs) were 0.40 (0.17-0.90) for use of HRT >3 years and 0.67 (0.47-0.94) for ever use of OCs, compared with never use. In **Chapter Two**, a prospective analysis of composite lifestyle factors and gastric cancer risk further elucidated that composite scores representing healthy lifestyles were significantly associated with reduced risk of gastric adenocarcinoma in a dose-dependent manner. HRs (95% CIs) for total, cardia, and non-cardia gastric adenocarcinoma for the highest (score 5) versus lowest composite score (score 0/1/2) were 0.42 (0.31-0.57), 0.22 (0.10-0.47), and 0.55 (0.39-0.78), respectively (all P_{trend}<0.001). These findings are very encouraging for a comprehensive strategy for promoting healthy living that could be effective for primary prevention of gastric cancer even in populations with a relatively high background risk of gastric cancer and high prevalence of *H. pylori*. No prospective evidence was available to elucidate the association between extreme telomere length

and gastric cancer risk. In **Chapter Three**, we conducted a prospective analysis found that both extreme short (1^{st} vs. 2^{nd} quintile: HR=1.63, 95% CI: 1.08-2.47) and long (5^{th} vs. 2^{nd} quintile: HR=1.55, 95% CI: 0.97-2.47) telomere length is associated with increased risk of gastric adenocarcinoma. The public health significance of these findings is the identification of novel modifiable risk factors among certain population subgroups with high risk of gastric adenocarcinoma and paving way for future preventative strategies.

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PREFACE

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I thank Siew-Hong Low of the National University of Singapore for supervising the field work of the Singapore Chinese Health Study and the Singapore Cancer Registry for assistance with the identification of cancer outcomes.

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1. INTRODUCTION

1.1 GASTRIC CANCER WORDWIDE INCIDENCE AND TIME TREND

Gastric cancer poses a heavy public health burden globally. It is the fifth most commonly diagnosed malignancy worldwide with 951,594 incident cases (age-adjusted incidence rate: 12.1 per 100,000) and the third leading cause of cancer deaths with 723,073 deaths (age-adjusted mortality rate: 8.9 per 100,000) based on the 2012 estimates by International Agency for Research on Cancer (IARC)[1]. More than 70% of incident cases developed in developing countries. Higher incidence rates were observed in South Korea, Japan, China, and Latin American and Eastern European countries. About 50% of incident cases occurred in Eastern Asia[2]. For example, gastric cancer was the seventh most common cancer among male Chinese (age-adjusted incidence rate: 10.7 per 100,000) and the ninth most common cancer among female Chinese in Singapore (6.8 per 100,000)[3].

The past four decades have witnessed the declining of gastric cancer incidence especially among high risk regions such as Japan, China and Singapore with an average 50% reduction in rate[2]. Although the prognosis of patients with gastric cancer has greatly improved; the 5-year age-adjusted relative survival rate of gastric cancer though improved by as far as 50% from 14.3% in 1975 to 28.8% in 2007 in the United States, but still remained lower compared with other cancer sites such as colorectal (64.9%) and breast (89.4%) cancer. In high risk regions such as Eastern Asia, the survival rate ranged from less than 20% for less developed countries to around 31-58%

for China, Japan and South Korea. For developed countries in high risk regions such as Japan, gastric cancer patients had a better prognosis with 54% 5-year survival rate due to early detection[4].

1.2 GASTRIC CANCER RISK FACTORS

1.2.1 Helicobacter pylori

Helicobacter pylori (*H. pylori*) infection was the strongest environmental risk factor for non-cardia gastric cancer development accounting for 75% of prevalent cases worldwide[5]. The prevalence of *H. pylori* infection varies substantially worldwide with the highest rate of 80% in high-risk area for gastric cancer such as Eastern Asia and the lowest rate of 30% in Western Europe[6]. *H. pylori* infection has also been classified as group I carcinogen by IARC since 1994[7]. A meta-analysis of 12 nested case-control studies in the prospective cohort has shown that *H. pylori* infection was associated with more than doubled risk of gastric cancer (OR=2.36, 95%CI: 1.89-2.81)[8]. When the results were stratified by cardia versus non-cardia anatomical type, the association only appeared among non-cardia cases (OR=2.97, 95%CI: 2.34-2.77) and became even stronger for samples collected more than 10 years before cancer diagnosis (OR=5.93, 95%CI: 3.41-10.30)[8]. There is possible biological plausibility explaining why H. pylori infection was not associated with cardia gastric cancer risk. *H. pylori* infection causing atrophy gastritis leads to reduction in gastric acidity and further alleviate the extent of gastroesophageal reflux disease (GERD)[8]. It has been well established that GERD was associated with risk of developing esophageal and cardia gastric adenocarcinoma[9]. The CagA *H. pylori* was the most investigated marker for virulence and possesses cag pathogenicity island[5]. CagA positive strain types of *H. pylori* was associated with an elevated risk of gastric cancer than CagA negative strain types based on a meta-analysis of 16 prospective cohort and case-control studies (OR=2.01, 95% CI: 1.21-3.32)[8]. The underlying biological mechanisms for *H. pylori* increasing risk of non-cardia gastric

cancer include overexpression of growth factors such as epidermal growth factor receptor (EGFR), methylation of cellular adhesion genes such as E-cadherin (CDH1), inactivation of tumor suppressor genes such as p53 and runt-related transcriptional factor 3 (RUNX3) and initiation of chronic inflammatory responses increasing pro-inflammatory cytokines levels such as IL-8 and TNFα[6].

1.3.2 Environmental risk factors

Tobacco smoking is the leading environmental risk factor for gastric cancer development after *H. pylori* infection. Since 2004 it has been classified as group I carcinogen by IARC[10]. A meta-analysis of 27 cohort studies showed a 62% increased gastric cancer risk for current versus never smokers among males (RR=1.62, 95% CI: 1.50-1.75) and 20% among females (RR=1.20, 95% CI: 1.01-1.43)[11]. There are more than 70 known carcinogens in cigarette smoke including tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons (PAH)[12]. Tobacco smoke also contains high level of nicotine which could promote carcinogenesis. *In vitro* studies showed that nicotine could activate nicotinic acetylcholine receptors (nAChRs) and induce cellular proliferation in gastric cancer cell lines by upregulating cyclooxygenase 2 (COX-2)[13]. It could also increase phosphorylating extracellular signal-regulated kinase-1/2 (ERK1/2) to further activate the downstream signaling pathways involving COX-2 and ERK[14]. Previous study also found that CagA positive *H. pylori* infection acts synergistically with cigarette smoking to increase risk of non-cardia gastric cancer. The risk of gastric cancer increased by almost 16-fold for current smokers and infected with CagA positive *H. pylori* compared with non-current smokers who were not infected[15].

Heavy alcohol consumption is associated with elevated gastric cancer risk. In human body, ethanol is metabolized to acetaldehyde, a group I carcinogen classified by IARC, and further

oxidized into nontoxic acetate[16]. In *vivo* studies have shown an increased incidence of stomach adenocarcinoma in rats administered with the highest acetaldehyde concentration compared with the lowest level[16]. A review of 15 cohort and 44 case-control studies found that heavy drinkers (≥ 4 drinks/day) had a 20% higher gastric cancer risk than nondrinkers (RR=1.20, 95% CI: 1.01-1.44)[17]. A possible explanation is that alcohol could damage gastric mucosa layer and enhance *H. pylori* adherence to gastric mucosa, as higher alcohol consumption increased *H. pylori* infection[18].

Mounting evidence showed that high intake of red meat or processed meat could increase gastric cancer risk. Based on a review of 12 cohort and 30 case-control studies, both red meat and processed meat increased gastric cancer risk by 45%[19]. Heme iron intake from red meat could endogenously convert into carcinogens such as *N*-nitroso compounds (NOCs) as study showed a highly significant correlation between heme iron from red meat and endogenous NOCs formation[20]. In the EPIC cohort, highest heme iron intake level from red meat was found to be associated with a 67% increase in gastric adenocarcinoma risk (HR=1.67, 95% CI: 1.20-2.34) compared with the lowest level[21]. For fruits and vegetables consumption, a meta-analysis of 24 cohort studies found a significant 10% reduction in risk of gastric cancer associated with highest levels of fruit (RR: 0.90, 95% CI: 0.83-0.98) and vegetables using a validated assessment method (RR: 0.90, 95% CI: 0.79-1.01)[22]. Antioxidants such as vitamin C in fruit and vegetables can inhibit the endogenous NOC formation. Also carotenoids, vitamin C and E in fruits and vegetables cast effect on DNA methylation modulation, induce detoxification of phase II enzymes and promote cellular apoptosis[23, 24].

Sodium intake and gastric cancer association have been extensively studied in different populations. Two *in vivo* studies in rats showed that administration of high concentration of sodium chloride (1.3-4.5 M) caused immediate damage to gastric mucosa, increased cellular proliferation,

and altered the viscosity of gastric mucosa[25, 26]. A review of 11 cohort studies found a significant association between highest level of salt intake and gastric cancer risk, especially in Japan (RR ranged from 2.2-5.4) where salt intake was much higher than elsewhere[27].

Obesity could increase the risk of gastro-esophageal reflux diseases (GERD) which is associated with high risk of esophageal and gastric cardia adenocarcinoma[28]. Overweight and obesity (BMI≥25) increased gastric cancer risk by 20% based on a review of 10 cohort studies (6 from non-Asian populations and 4 from Asian populations) (OR=1.22, 95% CI: 1.06-1.41) and especially for gastric cardia cancer (OR=1.55, 95% CI: 1.31-1.84)[29]. High BMI and increased gastric cancer risk was biologically plausible. Pro-inflammatory cytokines produced from excess body fat could lead to chronic inflammation. *In vitro* study showed that overexpression of interlukin-1β could lead to inflammation in gastric cells and eventual carcinoma development[30]. Also excess body fat could upregulate insulin-like growth factor-1 (IGF-1) which stimulates cellular proliferation and inhibit apoptosis[31].

1.3 PROPOSED GASTRIC CARCINOGENESIS MODEL

Approximate 90-95% of gastric cancer cases are adenocarcinomas and originate from glandular epithelium of the gastric mucosa, while another 5-10% cases are non-Hodgkin's lymphomas and stromal tumors[32]. By anatomical sites, gastric cancer could be classified as cardia gastric cancer and non-cardia gastric cancer. An estimated 27.3% of incident gastric cancer cases in 2012 were cardia gastric adenocarcinoma[33]. Based on Lauren's classification, there are two main histological types of gastric cancer: intestinal and diffuse type. Intestinal type gastric cancer is more related with dietary and environmental risk factors and eventually develop into gland-like structures through multistep progression[34]. On the other hand, diffuse type gastric cancer was less common accounting for 15% of all cases in the US[35]. Diffuse type gastric cancer lacks glandular

structures and premalignant lesions, more poorly differentiated and has worse prognosis than intestinal type gastric cancer[34]. The Correa's model was the most widely used gastric cancer pathology model to define the precancerous lesions development for intestinal type gastric cancer. It postulated that the intestinal type of gastric cancer was the end result of progressive changes in the gastric mucosa, starting with chronic gastritis, followed by multifocal atrophic gastritis (MAG) and intestinal metaplasia (**Figure 1**). *H. pylori* infection plays an essential role in this model and CagA positive *H. pylori* strain type was oncogenic and was associated with a higher risk of developing gastric pre-neoplastic lesions[36].

1.4 IDENTIFICATION OF GASTRIC CANCER RISK FACTORS

Worldwide gastric cancer incidence rates are twice as high in men than in women[5, 33]. While lower exposure to risk factors such as cigarettes smoking and alcohol consumption among women may partly explain the lower incidence rate of gastric cancer, higher lifetime exposure to estrogens may also contribute to their overall lower risk of gastric cancer. This notion is also supported by the fact that the male to female ratio in gastric cancer incidence rates peaks at 2.5 around age 60 and then declines to as low as 1.5 after age 60, suggesting a diminishing protective effect for women during their post-menopausal year[37, 38]. Therefore, we hypothesize that 1) longer cumulative exposure to endogenous circulating female hormones, and 2) postmenopausal hormone use (such as hormone replacement therapy and oral contraceptives) are associated with decreased risk of gastric cancer (**Chapter 1**).

Several modifiable lifestyle factors have been identified as risk factors for gastric cancer. These risk factors are cigarette smoking[11], heavy alcohol consumption[17], obesity[29], high sodium intake[39], low physical activity[40], low vegetable and fruit intake[41] and high red meat intake[19]. People choose a lifestyle that would be determined by most, if not all, of these factors. Therefore, these

lifestyle factors are correlated with each other. People with more healthy lifestyle factors may have lower risk of gastric cancer than those with fewer factors if each of these factors provides an additional effect. Thus we hypothesized that a higher composite score of protective lifestyle factors would be associated with a lower risk of developing gastric adenocarcinoma (**Chapter 2**).

Telomeres are tandem nucleotide repeats of TTAGGG and locate at the end of eukaryotic chromosomes[42]. Telomeres play an important role in maintaining chromosome stability by preventing degradation, atypical combination, and chromosome ends fusion[43]. Progressive shortening of telomeres occur as a consequence of somatic cell divisions and is associated with increasing age[44]. Several other factors have also been identified to be associated with decreasing telomeres length including cigarette smoking[45] and oxidative stress[46]. When telomere shortens to a critical point, the cell undergoes cellular irreversible growth arrest, replicative senescence and apoptosis[47]. On the other hand, extreme long telomere length could indicate upregulation of telomerase, a reverse transcriptase enzyme helping maintain telomere length. Long telomere length thus increased chance of abnormality with occurrence of more cell divisions[48]. Though retrospective evidence indicated that extreme short and long telomere length were associated with increased risk of gastric cancer, no prospective evidence is currently available. Thus we hypothesized a U-shaped association between telomere length and risk of gastric cancer (**Chapter 3**).

2. CHAPTER ONE: FEMALES HORMONES AND GASTRIC CANCER

2.1 PREVIOUS STUDIES

Previous evidence from epidemiologic studies investigating reproductive factors and exogenous hormone use associations with gastric cancer risk support the notion that high level of estrogens may offer some protection against gastric cancer for women. A recent meat-analysis showed that longer menstrual cycling (i.e. window between menopause and menarche) of more than 39 years was associated with 26% reduced risk of gastric cancer compared with less than 27 years[49]. Women who reported ever use of hormone replacement therapy (HRT) had a statistically significant 23% reduced risk of gastric cancer[49]. However, these results were heavily weighted by findings from populations with low risk of gastric cancer and may not be generalized to high-risk populations. For example, HRT use was not associated with risk of gastric cancer in prospective cohort studies conducted in Japan[50] or China[51]. The lower prevalence of ever HRT use in Asians (e.g., 2% in Chinese women[51] compared with the 55% in the US[52]) may have contributed to a null association in Asian cohort studies. Thus we conducted statistical analyses among a prospective cohort study of Chinese in Singapore to evaluate whether associations were present between reproductive factors and/or exogenous hormone use and gastric cancer risk in a relatively high-risk population with a relatively high prevalence of exogenous hormone use, compared with other Asian populations.

2.2 METHODS AND MATERIAL

2.2.1 Study design and population

The Singapore Chinese Health Study (SCHS) was a prospective cohort study conducted among a total of 63,257 middle-aged and older (45-74 years) Singapore Chinese men and women between 1993 and 1998[53]. The participants were citizens or permanent residents of Singapore and spoke one of the two major dialect groups (Hokkien or Cantonese). At recruitment, baseline in-person interviews were conducted using a structured questionnaire for each participant to obtain demographics, body weight and height, tobacco smoking and alcohol consumption, physical activity, diet, medical history and family history of cancer. A validated, semi-quantitative food frequency questionnaire (FFQ) including 165-food items was used to assess the subject's usual in the past 12 months. For women only, a specific section of the questionnaire asked for histories of reproductive factors (i.e., age at first menstrual period, menopausal status, age at last menstrual period, age at first birth, and number of births), use of oral contraceptive (OC) pills (age at starting to use, total number of years used), HRT (age at starting to use, total number of years used), and history of hysterectomy (with or without ovaries removed). In the first (FU1) and second (FU2) follow-up telephone interviews that took place in 1999-2004 and 2006-2010, respectively, menopausal status and HRT use were updated. Ninety-one percent of female cohort participants who were alive completed FU1 survey that updated information on menopausal status, use of exogenous hormones and other lifestyle factors. The FU2 survey was completed on 82% of women who completed FU1 and were alive that provided another update on menopausal status and use of exogenous hormones. The current study included 34,022 female participants of the Singapore Chinese Health Study (SCHS). This study

was approved by the Institutional Review Boards at the National University of Singapore and the University of Pittsburgh (Pittsburgh, PA).

2.2.2 Case ascertainment

All incident gastric cancer cases among the SCHS participants were identified through the linkage analysis with The Singapore Cancer Registry under the National Registry of Diseases Offices (NRDO) of Singapore. Starting from 1968, this national cancer registry provides information on cancer patterns and trends in Singapore and has been shown to be comprehensive in recording of incident cancer cases[54]. Gastric cancer was defined using the International Classification of Disease Oncology, 3rd edition (ICD-O-3) as C16.0-C16.9. As of December 31, 2013, 315 gastric cancer cases had been identified among female participants of SCHS. Among those identified cases, 269 adenocarcinoma (8140/3-8560/3) cases were included in our final analysis. Excluded cases included 18 sarcomas (8800/3-8936/3), 14 lymphomas (9590/3-9699/3) and 14 unspecified histology types.

2.2.3 *H. pylori* and chronic gastritis infection status

To further study the potential associations with years of menstrual cycling, HRT and OC use for gastric cancer risk while adjusting for *H. pylori* infection and chronic atrophic gastritis status, a nested matched case-control study was conducted among 187 female participants. Details of this sub-study have been previously reported[55]. A total of 48 female gastric cancer cases and 139 controls matched on age at study enrollment (within 3 years), father's dialect and date of sample collection (within 6 months) were included in this analysis. Three *H. pylori* antigens including CagA (116 kD), VacA (89 kD), and UreA (30 kD) were quantified using a validated assay to determine subject's *H. pylori* past and current infection status[56]. Plasma pepsinogen I (PG I) and II (PG II) were measured to define atrophic gastritis status using cutoff

points of PG I<70 ng/mL and PG I:II ratio <3, as recommended by the manufacturer (LZ Test "Eiken" Pepsinogen I and II, Tokyo, Japan).

2.2.4 Statistical analysis

Cox proportional hazards regression models were performed to calculate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) to examine the associations between exogenous hormone use (HRT and OC) and reproductive factors, and gastric cancer risks among all 34,022 female participants. Person-years of follow-up were computed from the enrollment date to the date of gastric cancer diagnosis, death, migration out of Singapore, or December 31, 2013, whichever occurred first. The proportional hazards assumption was examined by testing the significance of Pearson's correlation coefficient between Schoenfeld residuals of exogenous hormone use and ranked survival time. We found no violation of proportional hazards assumption.

HRT use was defined as ever taking estrogens with or without progesterone for the purpose of alleviating symptoms of menopause or other reasons, and was determined based on the information reported at baseline and two follow-up surveys. **Figure 1** depicts the algorithm used to estimate the total duration of HRT use. The person-years of follow-up for the HRT use model was calculated from the date of the most recent questionnaire (i.e., baseline, FU1 or FU2) to the censor date, as described above.

To estimate the cumulative duration of exposure to endogenous sex steroid hormones, we calculated the number of years of menstruation in the following manner:

[(Age at menopause or baseline interview for premenopausal women)-(Age at menarche)]-[(9/12)(Number of Births)]-(years of OC use)*

Age at menopause and type of menopause were ascertained at the baseline survey. Age at menarche, age at natural menopause, and quartile categories of menstrual cycling years were

used in multivariable models to investigate their associations with gastric cancer risk. In addition, the following reproductive factors were evaluated in relation to gastric cancer risk: parity (nulliparous, parous), age at first birth (≤20 years, 21-25 years, 26-30 years and ≥31 years) among parous women, number of births (0, 1-2, 3-4, ≥5), and combined oophorectomy and hysterectomy status (no oophorectomy or hysterectomy, hysterectomy with no ovary removed, oophorectomy with or without hysterectomy).

Covariates selected as potential confounders for the present analysis were those that had been previously reported to be associated with gastric cancer risk in our study population[55] or those that were associated with any HRT and/or OC use and gastric cancer risk (both *P* values<0.10) in addition to factors related to the selection and enrollment of study subjects. The final set of covariates included in the multivariable regression models were age at interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), body mass index (in kg/m^2), educational level (no formal education/primary education and ≥secondary education), smoking status (never/former and current), daily coffee intake (non-daily drinkers and daily drinkers), and sodium intake (in mg/day). Adjustment for additional variables including intake of fruit or vegetables, or history of gastric/duodenal ulcer did not materially change the association between reproductive factors or exogenous hormone use and gastric cancer risk. Thus these results are not shown in the present report. BMI was evaluated as a potential effect modifier on the relationship between selected exposures and risk of gastric cancer by conducting stratified analyses by BMI, and by evaluating whether an interaction term (e.g., hormone use x BMI) was statistically significant in the multivariable model.

To examine the association between reproductive factors or exogenous hormone use and gastric cancer risk with the adjustment for *H. Pylori* infection status, a nested matched case-

control study design was used based on the available measurement of serological status of *H. pylori* on the selected cases and controls only. To maximize the number of subjects included in the analysis, unconditional logistic regression was used to calculate odds ratios (ORs) and their corresponding 95% CIs for gastric cancer for all participants and for participants with positive *H. pylori* infection only, which was defined by the serologic status of CagA. Other serological biomarkers such as VagA and UreA did not add much information on the determination of *H. Pylori* infection status, thus these two serological biomarkers were not included. Included in the models were covariates for the matching factors (age at baseline, dialect group, and date of biospecimen collection), as well as interview year, cigarette smoking, body mass index, coffee intake, and sodium intake. Further adjustment for atrophic gastritis status did not materially alter the ORs for gastric cancer among participants with positive CagA status, therefore these results are not shown.

All statistical analyses were performed in SAS 9.3 software package (SAS Institute, Inc.). All *P* values reported were two-sided. *P* values <0.05 were considered to be statistically significant.

2.3 RESULTS

At baseline, study participants had a mean age of 56.3 years [standard deviation (SD)=8.0]. Women who reported ever HRT and/or OC use were younger, had higher intake of sodium, higher level of education, were less likely to smoke cigarettes, and less likely to have menopause by natural means (**Table 1.1**). Risk of gastric cancer was inversely associated with level of education (≥secondary level versus <secondary level: HR=0.79, 95% CI: 0.54-1.17) and positively associated with current versus never/former smoking status (HR=1.41, 95% CI: 0.94-2.11) and body mass index (≥28 versus <20 kg/m^2: HR=1.35, 95% CI: 0.81-2.26). The

association with daily alcohol consumption was very imprecise (daily versus nondrinker: HR=1.28, 95% CI: 0.47, 3.43).

The associations between reproductive factors and gastric cancer risk are shown in **Table 1.2**. Age at menarche and age at first birth were not associated with risk of gastric cancer. Parity was inversely associated with gastric cancer risk, and a non-linear trend was observed with number of children, compared with nulliparous women. History of hysterectomy with or without oophorectomy was not associated risk of gastric cancer whereas history of oophorectomy regardless of hysterectomy was associated with a reduced, but statistically non-significant association for gastric cancer. Compared with natural menopause, other non-natural type was associated with a 52% reduction of gastric cancer risk. Further adjustment for ever HRT and/or OC use did not change the association (HR=0.51; 95% CI: 0.28-0.92 for non-natural versus natural menopause). Among women who reported having menopause by natural means, increasing age at menopause was associated with a decreased risk of gastric cancer (*P* for trend = 0.04); women who had natural menopause at 55 years or older had a 50% lower risk of gastric cancer compared to those with menopause before 45 years of age. Overall, the highest quartile range of menstrual cycling years was associated with a 33% reduced risk of gastric cancer among premenopausal and natural postmenopausal women compared with the lowest quartile range though there was no evidence for a trend.

The associations between use of HRT and OC and risk of gastric cancer are shown in **Table 1.3**. HRT use was associated with a 28% reduced risk of gastric cancer compared with never use. The reduced risk was more pronounced for women who used HRT for more than 3 years. Similarly, ever use of OC was also associated with a statistically significantly reduced risk of gastric cancer (HR = 0.67, 95% CI = 0.47-0.94). The mutual adjustment for HRT and OC use

did not materially change the observed association between use of HRT or OC and gastric cancer risk (data not shown). Further adjustment for menopausal type (i.e., non-natural versus natural), the associations with HRT use remained (HRT ever versus never use, HR=0.73; 95% CI: 0.43-1.24; and HRT use >3 years versus never use, HR=0.40; 95% CI: 0.18-0.92).

The association for gastric cancer risk in relation to exogenous hormone use and total number of years of menstrual cycling over lifetime was further examined among a subset of study subjects with available measurement of *H. pylori* infection (CagA) status (**Table 1.5**). Similar to the results based on the entire cohort, a reduced risk of gastric cancer was observed for ever versus never use of exogenous hormones (HRT and/or OC) in all subjects as well as in those with positive CagA of *H. pylori* of this subset, although the inverse association did not reach the statistical significance level due to small sample size. For years of menstrual cycling, a statistically significant inverse association was observed for the third versus first quartile. The inverse association was still present, but no longer statistically significant among those positive for *H. pylori*.

2.4 DISCUSSION

In prospective analyses using data from a population-based cohort of Chinese in Singapore, we reported statistically significant inverse associations for gastric cancer risk with greater years of menstrual cycling, non-natural cause of menopause, older age at natural menopause, and hormone use. The strongest inverse association was for more than 3 years of HRT use, with a statistically significant 60% risk reduction, compared with never use. Results from prospective cohort studies among populations with low background risk of gastric cancer incidence support an inverse association between HRT use and gastric cancer risk[52, 57]. Our findings, among a population with a high background risk of gastric cancer, are consistent with

previous studies and lend further support for the notion that longer exposure to high circulating estrogens confers a reduction in gastric cancer risk.

In a meta-analysis conducted with results from five prospective cohort studies and two case-control studies, ever HRT use was associated with a statistically significant lower gastric cancer risk (summary RR=0.77, 95%CI: 0.64-0.92)[49]. Results from two Asian cohorts [51, 58] were included, but their risk estimates contributed only 8.6% weight to the summary RR, compared with 74% weight from the US and European cohort results. In the only Asian prospective cohort study that evaluated HRT use and gastric cancer incidence, no association was observed with ever use[51]. However, the prevalence of HRT use among participants of that cohort was only 2%[51]. In the present study, 12.6% reported ever HRT use. With a higher prevalence of HRT use, the present study provided greater statistical power for us to evaluate and detect a modest effect of HRT use on gastric cancer risk.

Our finding for a statistically significant inverse association between OC use and gastric cancer risk has not been observed in other studies. Previous studies reported a positive[57], inverse[59], and null[51, 52] association between OC use and risk of gastric cancer in various populations. While a possible chance finding, there is biologic plausibility for a protective effect of OCs on gastric cancer development. OCs inhibit ovulation in part by decreasing production and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone[60, 61]. In rats, administration of FSH resulted in increased markers of oxidative stress[62]. Reactive oxygen species can result in genomic DNA damage that initiates and/or promotes carcinogenesis[63, 64]. Accumulation of oxidative DNA damage has been elevated in tissues of the stomach of patients with intestinal metaplasia, a precursor of gastric cancer[65]. There remains, however, a lack of

direct evidence between OC use and decreased gastric tissue-specific oxidative stress in humans. Future studies are warranted to confirm our findings.

We evaluated the relationship between reproductive factors and gastric cancer risk, because factors, such as later age at menopause and years of menstrual cycling not only are associated with increased levels of circulating estrogens and/or their metabolites[66], but also represent longer period of cumulative exposure to sex hormones[67]. Thus, these factors may be associated with lower gastric cancer risk. We reported statistically significant inverse associations for gastric cancer risk with greater years of menstrual cycling (>34.4 versus ≤28.4). Our results for menstrual cycling years are consistent with results from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, where a statistically significant inverse association between years of menstrual cycling and gastric cancer risk (>37 versus <27 years: HR=0.55, 95% CI: 0.31-0.98) was reported[57].

We also reported statistically significant inverse associations for gastric cancer risk with non-natural cause of menopause and with older age at natural menopause (≥55 versus <45 years). The mean age at menopause (i.e., 49 years) in our cohort was similar to European (i.e., 50 years) and U.S. (i.e., 49 years) populations [68, 69]. Compared with women who had a natural menopause, those who had non-natural menopause had a younger age at menopause and were more likely to be users of HRT and/or OC, which could have masked an inverse relationship in the previous studies that combined women with different menopausal types and reported no clear relationship [52, 57]. In our study, when women with non-natural cause of menopausal were included in the analysis, we did not observe a statistically significant association between age at menopause and gastric cancer risk (data not shown). Although the inverse association with non-natural menopause was only slightly attenuated after adjusting for age at menopause and

hormone use (HR=0.45; 95% CI: 0.25, 0.83), the finding should be interpreted cautiously. It was not possible to completely remove the potential confounding effects of age at menopause or HRT use, or other, unmeasured factors on the menopause type-gastric cancer association. In addition, our finding was inconsistent with the results of no association [50, 52] and a positive association[51] that have been reported for surgical menopause.

Our findings of no association for gastric cancer risk was observed with age at menarche or age at first birth were consistent with findings from previous epidemiologic studies[49]. The mean age at menarche (i.e., 14.4 years) in our cohort was slightly older compared to European (i.e., 13.0 years) and U.S. (i.e., 12.7 years) populations [68, 70] Compared with nulliparous women, having three to four children was inversely associated with gastric cancer, but no association was seen for having 5 or more children. Most previous prospective studies have not observed associations with parity or a trend with increasing number of births[57, 71]. Having more full-term births is not directly related to circulating estrogens[72], so if having children protects against gastric cancer, the mechanism is not likely to be hormonally driven.

An important role of estrogens in gastric carcinogenesis has been documented in animal studies that show lower markers of cell proliferation and lower gastric cancer incidence following carcinogen administration in male versus female rats[73, 74]. 17β-Estradiol can suppress the pathologic changes, such as atrophy, hyperplasia, intestinal metaplasia and dysplasia in gastric epithelium of mice infected with *H. pylori*[75]. A hypothesized biologic mechanism for such a protection includes increased expression of the trefoil factor family (TFFs) proteins[76], which are expressed in the normal gastric mucosa[77, 78]. In addition, their expression is increased around the site of mucosal injury and is shown to contribute to mucosal repair by promoting epithelial restitution[79, 80]. TFF1 expression in estrogen-receptor positive breast cancers was

positively correlated with plasma estradiol levels in postmenopausal patients[81]. In contrast, TFF3, also known as intestinal trefoil factor (ITF) is not expressed in normal gastric mucosa and instead is expressed by goblet cells in areas of intestinal metaplasia. ITF expression is reported in 55% of all gastric cancers, and is correlated with aggressive phenotype [82]. Serum levels of TFF3 are associated with later stage at diagnosis of gastric cancer (P=0.002), and are higher among patients compared with unaffected controls (P<0.0001) [83]. The relationship between estrogen and TFFs in the gastrointestinal tract are unclear, but may shed light on the protective mechanisms behind the lower risk of gastric cancer observed in women, compared with men.

The strengths of our study include its large sample size of over 34,000 participants and relative long follow-up, with a mean of 16 years. The prevalence of ever HRT use was low (around 3.7%) at baseline and more women started to use HRT at later age after menopause. By incorporating the follow-up information, 12.6% of the study participants reported ever HRT use at either baseline, FU1 or FU2. Our ability to update status during follow up likely provided more accurate measures of exposure than if we had relied only on information collected at baseline. Our study had more statistical power to detect a possible inverse association between exogenous hormone use and gastric cancer risk compared with previous studies conducted in populations with high gastric cancer incidence. Pre-diagnostic biospecimens were available among a subset of our samples allowing for further investigating the exogenous hormone use-gastric association among participants with atrophic gastritis and positive *H. pylori* infection status.

Our study also has several limitations. We did not have information on specific types and dosage levels of HRT and OC used by the study participants. There could be potential variations of exogenous estrogen exposure level between participants even if they have similar duration of

use. This could lead to non-differential misclassifications and possibly bias the results towards null. Second, we applied an algorithm to estimate total duration of HRT use. It is possible that we could have under or overestimated duration of HRT use, but any misclassified duration calculations are likely to be random, which would typically attenuate the HRT-gastric cancer risk association.

Greater years of menstrual cycling, older age at natural menopause, non-natural menopause, and hormone use are associated with a statistically significant decrease in gastric cancer risk in a prospective cohort of Chinese women in Singapore. Our results support the notion that an underlying estrogenic mechanism is responsible, in part, for the lower incidence of gastric cancer in women compared with men. Future prospective studies are needed to verify our findings in other populations with a high background risk of gastric cancer.

3. CHAPTER TWO: COMPOSITE LIFESTYLE FACTORS AND GASTRIC CANCER

3.1 PREVIOUS STUDIES

There have been only two prospective cohort studies that examined the association between combined lifestyle factors and risk of gastric cancer; both of them were conducted in the European countries where incidence rate of gastric cancer is lower (9.4 per 100,000) than the world average (12.1 per 100,000)[1]. In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, a composite index of three healthy lifestyle factors was associated with a statistically significant reduced risk of gastric adenocarcinoma[84]. The other study in France with a composite index of five lifestyle habits was also associated with a reduced risk of cancer of digestive system including the esophagus, stomach, biliary tract, small bowel, and pancreas[38]. To our knowledge, there has been no study investigating the composite score of multiple lifestyle factors in relation to risk of gastric cancer in Asian populations, which are at moderate to high risk for gastric cancer. The findings from low-risk European populations may not be applicable to moderate- to high-risk Asian populations. Therefore we conducted an analysis for the associations between composite score of lifestyle factors consisting of cigarette smoking, alcohol consumption, BMI, dietary pattern and sodium intake, and risk of gastric adenocarcinoma in a prospective cohort of Chinese men and women in Singapore who were at medium risk of gastric cancer (11.9 per 100,000)[3].

3.2 METHODS AND MATERIAL

3.2.1 Study design and population

The Singapore Chinese Health Study (SCHS) was a prospective cohort study conducted among a total of 63,257 middle-aged and older (45-74 years) Singapore Chinese men and women between 1993 and 1998[53]. The participants were citizens or permanent residents of Singapore and

spoke one of the two major dialect groups (Hokkien or Cantonese). We excluded 1,936 subjects who had a history of cancer at baseline. Thus the present analysis included 61,321 participants. The SCHS was approved by the Institutional Review Boards at the National University of Singapore and the University of Pittsburgh (Pittsburgh, PA).

3.2.2 Case ascertainment

Gastric cancer cases among the SCHS participants were identified through the linkage analysis with The Singapore Cancer Registry under the National Registry of Diseases Offices (NRDO) of Singapore. This nationwide registry has collected information on individual cancer patients and national cancer trends and patterns since 1968 [3] and has been shown to be comprehensive in recording incident cancer cases[16]. Gastric cancer was defined using the International Classification of Disease Oncology, 3rd edition (ICD-O-3) as C16.0-C16.9. By December 31, 2014, a total of 801 incident cases of gastric cancer were identified among all participants of SCHS. Among them, 32 were sarcomas, 29 lymphomas and 49 unspecified histology types cases were excluded. Thus the present study included 691 cases of gastric adenocarcinoma, of which 118 were cardia, 491 were non-cardia and 82 site unspecified cases.

3.2.3 Assessment of lifestyle factors and composite lifestyle score

For all study participants, baseline interviews between 1993 and 1998 were conducted in person using a structured questionnaire to elicit subjects' information on age, height, weight, level of education, tobacco use, physical activity, medical history and usual adult diet including alcohol consumption. The structured semi-quantitative food frequency questionnaire (FFQ) listed 165 dietary items that represented all major Chinese food and beverage items in Singapore. For each dietary item, each subject was asked to choose a consumption frequency among 8 pre-defined categories from "never or hardly ever" to "two or more time a day" along with a portion size of

the item assisted with a food photo album. This FFQ was validated subsequently in a sub-cohort of our study population[53].

For cigarette smoking, each ever smoker was asked the following questions: “What is the average number of cigarettes that you smoked per day?” and “What is the total number of years that you smoked cigarettes on a regular basis?” Total number of pack-years of smoking was calculated as the number of packs (20 cigarettes per pack) smoked per day multiplied by the number of years of smoking.

For daily ethanol consumption, participants were asked “How often did you drink each type of beverage including beer, rice wine, grape wine and hard liquor during the past year?” and “What was the usual serving size of this type of beverage?” Daily consumption of each type of alcoholic beverage was calculated by frequency multiplied by the serving size. Thus total daily ethanol consumption was the sum of ethanol over all types of alcoholic beverages consumed. The average ethanol content is 13.5 g in one drink (375 ml) of beer, 10.85 g in one drink (30 ml) of rice wine or hard liquor, and 11.68 g in one glass (118 ml) of grape wine.

Daily sodium intake was derived from validated FFQ and the Singapore Food Composition Table that also provides sodium content for each of 165 dietary items (mg per 100 g). The final value of sodium intake was adjusted for total energy intake (mg per 1,000 kcal).

Dietary pattern was determined using the principal component analysis (PCA) as described previously[85]. Briefly, we identified two dietary patterns among SCHS participants: vegetable-fruit-soy (VFS) and meat-dim-sum (MDS). VFS was characterized by high intake of fruits, vegetables and soy foods whereas MDS by high intake of pork, chicken, dim-sum foods and noodle dishes, respectively. For each subject, the average of the two VFS (ascending order) and MDS (descending

order) ranking scores (range 1 to 100) was calculated for the present analysis, a high score was indicative of a high intake of VFS and a low intake of MDS (**Table 2.6**).

Two sets of algorithm were applied for construction of composite protective lifestyle score based on dichotomized cutoff and Z-score of each factor. In the first algorithm, each lifestyle factor was dichotomized and final cutoff value was chosen to reflect the strongest effect size for its univariate association with gastric adenocarcinoma risk. Pack-years of smoking was categorized at median (i.e., 21.9) among ever smokers (low risk/protective lifestyle score **1**= <21.9, **0**= ≥21.9). Daily ethanol consumption was categorized at 8.1 g (the third tertile) among ever drinkers (**1**= <8.1, **0**= ≥8.1). Dietary pattern score was categorized at 62 (the fourth quartile) of the entire cohort (**1**= ≥62, **0**= <62). Daily sodium intake was categorized at 782 mg per 1000 kcal (the third tertile) (**1**= <782, **0**= ≥782). BMI was categorized at ≥27.5 kg/m^2 for obesity recommended by the World Health Organization for Asian populations [86] (**1**= <27.5, **0**= ≥27.5 kg/m^2). The percentages of the study population in high or low score of these five individual lifestyle factors are presented in **Table 2.7**. The final composite protective lifestyle score was the sum of 5 individual lifestyle factors: represeted the lowest and 5 the highest protective lifestyle score.

The second algorithm was to generate sex-specific Z-score for each lifestyle factor to avoid over-fitting the model based on dichotomized values of individual lifestyle factors. In the study population, approximately 70% of participants were never smokers and 81% never consumed alcoholic beverages. Thus the Z-scores for pack-years of smoking and daily ethanol consumption were derived from ever smokers and ever alcoholic drinkers, respectively. We assigned half of the lowest Z-score for pack-years of smoking and daily ethanol to never smokers and never drinkers, respectively. The composite healthy lifestyle Z-score was the sum of Z-score for all five individual factors for each study subject as follows:

$$Composite\ Z\text{-}score = Z_{diet} - (Z_{smoking} + Z_{drinking} + Z_{BMI} + Z_{sodium}).$$

A higher composite Z-score stands for higher score presenting healthier dietary pattern, lower pack-years of smoking, lower ethanol consumption, lower BMI and lower sodium consumption. The distribution of single lifestyle factor by quartile of composite Z-score was shown in **Table 2.8**.

3.2.4 *H. pylori* infection status testing

To further examine the association of composite protective lifestyle score in combination with *H. pylori* infection status in relation to gastric adenocarcinoma risk, a nested case-control study was conducted involving 522 subjects (133 gastric adenocarcinoma cases and 389 individually matched controls) whose serological *H. pylori* infection status was determined by the presence or absence of CagA 116 kDa in serum.

3.2.5 Statistical analysis

Person-years at risk for each of 61,321 eligible subjects were computed from the date of enrollment to the date of gastric cancer diagnosis, death, migration out of Singapore, or December 31, 2014, whichever occurred first. Cox proportional hazard regression method was employed for calculation of hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) of gastric adenocarcinoma associated with individual lifestyle factors and both the composite protective lifestyle scores derived from dichotomized cutoff and Z-score of these lifestyle factors together. Test for liner trends was conducted by treating the composite lifestyle score as a continuous variable in the Cox model. The proportional hazards assumption was examined by testing the significance of Pearson's correlation coefficient between Schoenfeld residuals of the composite lifestyle score and ranked survival time[87]. We found no violation of proportional hazards assumption.

To adjust for potential confounding, age, sex, dialect group, year of recruitment, and level of education were included in all regression models. Further adjustment for other covariates such as intake of individual vegetables and fruit, family history of cancer and history of gastric/duodenal ulcer did not meaningfully alter the association between the composite lifestyle score and gastric adenocarcinoma risk. Thus the results presented were not adjusted for these variables.

Population attributable risk (PAR) estimate was computed for the proportion of gastric adenocarcinoma cases that would be avoided on a population level attributable to higher composite lifestyle category. Based on the HRs from Cox proportional hazard regression model and their variance-covariance matrix and the prevalence of each unique combinations of the covariates in the model, an algorithm developed by Spiegelman and colleagues was applied to calculate partial PAR along with its 95% CIs while age, sex, dialect group, year of recruitment, and level of education remain unchanged [88].

To examine the potential modifying effect of subclinical symptoms of gastric cancer on the association between composite protective lifestyle score and gastric adenocarcinoma risk, we conducted sensitivity analyses on subset of dataset divided by the length of follow-up, e.g. ≤5 years and >5 years. To investigate the association between composite lifestyle score and gastric adenocarcinoma risk with adjustment of *H. pylori* infection status, conditional logistic regression model was performed in the nested case-control study for all subjects and for subjects with positive *H. pylori* infection status defined by positive CagA test results. Stratified analyses were performed by anatomical sites such as cardia and non-cardia of the stomach.

All statistical analysis was performed in SAS 9.4 software package (SAS Institute, Inc.). All P values reported are two-sided. P values <0.05 were considered to be statistically significant.

3.3 RESULTS

With increasing composite lifestyle score, there was an increase in proportion of women and a decrease in BMI. A higher composite lifestyle score was also characterized by fewer current smokers, lower pack-years of smoking, and lower daily intake of ethanol, sodium and red meat (**Table 2.1**). The distributions of individual lifestyle factors across different composite score of protective lifestyle factors are shown in **Table 2.9**. These individual lifestyle factors were not or moderately correlated each other (all correlation coefficients < 0.23).

After more than one million cumulative person-years of follow-up (mean 16.9 years per subject), as of December 31, 2014, a total of 801 incident cases of gastric cancer were identified among all participants of SCHS. Among them, 32 were sarcomas, 29 lymphomas and 49 malignancies with unspecified histology; all of them were excluded from the present analysis. Thus the present study included 691 cases of gastric adenocarcinoma; among them, 118 were cardia, 491 were non-cardia and 82 were unspecified subsite of the stomach. The mean duration between baseline interview and the diagnosis of all gastric adenocarcinoma cases was 6.9 years (standard deviation = 3.9).

Individual scores of all five protective lifestyle factors separately were significantly associated with a 18-34% reduction in HR of gastric adenocarcinoma (**Table 2.2**). The association was stronger for BMI with risk of cardia than non-cardia cancer (**Table2.2**). HR and 95% CIs for individual risk factor (before dichotomized) and gastric adenocarcinoma risk are presented in **Table 2.10**. The cut-off value of each lifestyle factors was chosen based on their risk association with gastric adenocarcinoma for the creation of composite score. High composite score of protective lifestyle factors was significantly associated with reduced hazard ratio of gastric adenocarcinoma in a dose-dependent manner (**Table 2.2**). Compared with the lowest composite

scores using dichotomous cutoff (0-2), HRs (95% CIs) of gastric adenocarcinoma for composite scores of 3, 4, and 5 protective lifestyle factors were 0.68 (0.52-0.88), 0.51 (0.40-0.66), and 0.42 (0.31-0.57), respectively (*P* trend < 0.001). This association was stronger for cardia than non-cardia cancer (**Table 2.2)**. Based on the distribution of the composite score, we estimated that 48% of total gastric adenocarcinoma, including 72% in cardia and 43% in non-cardia, could be attributable to these five risk factors combined.

We also examined the association between Z-score of single lifestyle factor and gastric adenocarcinoma risk **(Table 2.11)**. High Z-score for individual lifestyle factors were associated with increased risk of gastric cancer except for dietary pattern, which was inversely associated the risk though of statistical significance.

When these Z-score were summed up after reversing the Z-scores for risk lifestyle factors (see Methods section), high composite Z-score was associated with statistically significant, reduced risk of gastric adenocarcinoma (**Table 2.2**). Although weaker than the composite score of the dichotomized lifestyle protective factors, the inverse association was strong and in dose-dependent manner, and present for both cardia and non-cardia cancers. (**Table 2.2**). This inverse association between composite score of protective lifestyle factors, either derived from dichotomized categories or Z-scores, and gastric adenocarcinoma risk was present in both men and women (**Table 2.3**) and for both short (≤5 years) and long (>5 years) duration of follow-up of the entire cohort (**Table 2.4**).

To take into account the impact of *H. pylori* infection on the observed risk association, we conducted similar analysis in a nested case-control study within the SCHS whose serological status of *H. pylori* infection was determined by the presence or absence of CagA in serum. There was a statistically significant inverse association between composite score of dichotomized lifestyle

factors and gastric adenocarcinoma risk among all subjects of the case-control study after adjustment for *H. pylori* infection status as well as among subjects with positive CagA status only. A similar inverse association was observed for both cardia and non-cardia cancer. For composite Z-score, the association slightly attenuated after adjustment for *H. pylori* infection status and among subjects with positive CagA status, especially for non-cardia cases, given the small sample size (**Table 2.5**).

3.4 DISCUSSION

The present study demonstrates that a high composite score of five healthier lifestyle factors including smoking, alcohol consumption, BMI, a diet high in vegetables/fruit and low in red meat, and low intake of dietary sodium is significantly associated with reduced risk of developing gastric adenocarcinoma in an Asian population with high prevalence of *H. pylroi*. The highest composite score was associated with a statistically significant 58% decreased risk of gastric adenocarcinoma compared with the lowest composite score. These lifestyle factors together can account for up to half of the disease burden in this study population, of which approximately 85% had a history of infection with *H. pylori.*

To our knowledge, the present study is the first prospective study to examine the association between combined lifestyle factors and gastric adenocarcinoma risk in an Asian population with high *H. pylori* prevalence. There are only two previous reports, both in low-risk European populations, on the composite lifestyle factors and gastric cancer risk. In the EPIC study with 11.4 years of follow-up, highest score of 3 lifestyle factors (cigarette smoking, alcohol consumption, and adherence to a Mediterranean diet) was associated with a significant 50% decrease in risk of gastric adenocarcinoma [84]. The E3N (*Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale*) study in French women with 15 years of

follow-up showed similar results; the highest score of protective lifestyle factors, including abstinence from smoking, low to moderate alcohol drinking (less than 2 drinks/day), normal range of BMI (18.5-25 kg/m^2), high recreational physical activity and high vegetable and fruit intake, was associated with a statistically significant 40% decrease in risk of cancer in the digestive tract gastric cancer[38]. The findings of the present study are consistent with those in low-risk populations.

Z-score based lifestyle composite score is significantly associated with risk of gastric adenocarcinoma in a dose-response manner although its association was slightly weaker than the categorical based composite score. As a complementary approach to the categorical based composite score, Z-score method corroborates the inverse association between adaption of healthy lifestyle factors and reduced risk of gastric adenocarcinoma. Z-score based composite score was based on the standardized values for each factor with equal weight, which addressed the issue of data overfitting. However, this complete data-driven approach may not optimize the stratification of study subjects at risk, thus could lead to a weaker association between the summed Z-score and gastric cancer risk.

The strengths of our study include the prospective study design, unique study population (Southeast Asians), a relatively large sample size (63,000 participants), long-term follow-up (17 years) and serological status of *H. pylori* infection. A summary lifestyle factor score can classify individuals into more homogenous groups by their risk profile that minimizes potential misclassification and residual confounding effect. The main limitation is that all the information on lifestyle factors was self-reported at baseline with inherent non-differential misclassification, that could bias the risk estimates towards null. Therefore, the observed risk estimates may be lower than the true effect of these lifestyle factors on the risk of gastric adenocarcinoma. It should also be noted that our estimate of daily sodium intake may have missed some dietary sources, which

may cause some biased results. Given a prospective study design, both cancer cases and non-cancer individuals answered to the same dietary questionnaire. Thus, if there is any misclassification, it would be non-differential and lead to an underestimated risk association. Future studies using urinary excretion of sodium over 24 hours are warranted to confirm our findings.

In conclusion, we observed a strong, statistically significant association between high composite score of protective lifestyle factors and reduced risk of gastric adenocarcinoma. Altogether these factors can account for up to almost half of disease burden in this Asian population with a very high prevalence of *H. pylori*. These findings are very encouraging for a comprehensive strategy for promoting healthy living that could be effective for primary prevention of gastric cancer even in populations with a relatively high background risk of gastric cancer and high prevalence of *H. pylori.*

4. CHAPTER THREE: TELOMERE LENGTH AND GASTRIC CANCER

4.1 PREVIOUS STUDIES

Based on a meta-analysis of 16 case-control and 11 prospective studies, shortened telomeres were associated with cancer risk including bladder, esophageal, gastric, ovarian, head and neck, and renal cancer cases[89]. A more recent case-control studies on telomere length and gastric cancer risk among Han Chinese found a U-shaped association in which either extreme short or long telomere was associated with increased gastric cancer risk[90]. A potential explanation was that while shortened telomere could induce chromosomal instability initiating carcinogenesis, long telomeres on the other hand upregulate cell divisions and increase likelihood of abnormalities during the process and thus promote cancer development[48]. However, no association was found in prospective studies. A study on two prospective cohorts of over 40,000 participants found that telomere length was not associated with gastric cancer risk and death after gastric cancer diagnosis[91]. Therefore, findings from retrospective studies warrant further examination in a prospective study setting that could reduce potential selection bias and reverse causality.

Both *in vivo* and *in vitro* studies had shown that telomere length could be associated with micronutrients level involved in one carbon metabolism pathway such as vitamin B6, folate, and B12. Folate deficiency and high plasma homocysteine level were found to be related with lower telomere length among elderly males[92]. In a previous study in our study population, we observed a similar association between high plasma homocysteine and low leukocyte telomere length[93]. In a human cell study where folate deficiency condition was established, rapid elongation of telomere in short term followed by sudden shortening of telomere over long period was observed[94]. Meanwhile randomized interventional and observational studies from different populations also

demonstrated that dietary folate, vitamin B6, B12, or other one-carbon metabolites are positively associated with longer telomere length[95-97]. However, the interrelationship between dietary folate, telomere length and risk of gastric cancer remains unclear. We hypothesis that dietary folate, B6 or B12 intake could mediate the association between telomere length and risk of developing gastric cancer.

Therefore, we conducted a prospective analysis on participants in SCHS with blood sample collected to: 1) examine the role of telomere length in gastric cancer development; 2) whether this association differ by dietary folate, vitamin B6 or B12 intake level.

4.2 METHODS AND MATERIAL

4.2.1 Study design and population

Among 63,257 participants of the Singapore Chinese Health Study, a population-based prospective cohort of Chinese men and women aged 45-74 years recruited between 1993 and 1998, 28,219 provided baseline blood samples. The details of SCHS has been previously described[53]. Basically, the study participants were citizens or permanent residents living in the government housing estates and belonged to one of two major Chinese dialect groups (Cantonese and Hokkien). The present analysis included 26,540 (12,234 males and 14,306 females) subjects with available TSR values after excluding samples with insufficient DNA (n = 194) and/or patients with prevalent cancer at baseline blood draw (n = 1,465).

During baseline in-person interviews, a structure questionnaire was used for each participant to assess demographics, body weight and height, tobacco smoking, alcohol consumption, physical activity, diet, medical history and family history of cancer. Information on dietary consumption was obtained using a structured semi-quantitative food frequency questionnaire (FFQ) with 165 listed dietary items representing majority of food and beverage items

commonly consumed by the study population in Singapore. Dietary nutrients intake was derived based on the Singapore Food Composition Table designated for this study and has been validated with 24-hour dietary recall interview[53]. In the first follow-up telephone interviews (FU1) that took place in 1999-2004, information on body mass index (BMI), smoking status, number of cigarettes smoked per day and years of smoking were updated on 97% of included subjects.

4.2.2 Case ascertainment

Incident gastric cancer cases were identified from the Singapore Cancer Registry under the National Registry of Diseases Offices (NRDO) of Singapore by linkage analysis. Since 1968, the national cancer registry has provided information on cancer trend and patterns[98] and have been shown to be complete in recording of incident cancer cases[99]. Gastric adenocarcinoma was defined using the International Classification of Disease Oncology, 3rd edition (ICD-O-3) as primary site of C16.0-C16.9 and histology type of 8140/3-8560/3.

4.2.3 Telomere length measurement

Genomic DNA were extracted from leukocytes in peripheral blood samples by standard procedures and was stored at 4°C until analysis. Evidence had shown that human average leukocyte telomere length is highly correlated with telomere length in other tissues[100]. Quantitative polymerase chain reaction (PCR) method developed by Cawthon[101] was applied to quantify relative telomere length determined by the ratio of telomere repeat copy number (T) to single-copy gene for albumin (S) (i.e., TSR) on all subjects. In a random selected sample, TSR measurements showed high correlation with absolute telomere measurements using Western blot method (r=0.91). Each 10 μl experimental sample contained 20ng of DNA diluted in pure water and was aliquoted into the reaction well of a 96-well plate compatible with the Bio-Rad MyiQ Single Color Real-Time PCR Detection system. Standard DNA curve was drawn based on five concentrations

(1.85-150 ng/μl by 3-fold incremental increase) of reference DNA sample. All experimental DNA samples were assayed in duplicates. The mean percentage of coefficients of variability was 3.5%.

4.2.4 *H. pylori* infection status testing

To further examine the association of telomere length adjusting for *H. pylori* infection status in relation to gastric adenocarcinoma risk, a nested case-control study was conducted involving 522 subjects (133 gastric adenocarcinoma cases and 389 individually matched controls) whose serological *H. pylori* infection status was determined by the presence or absence of CagA 116 kDa in serum. The details of this case-control study have been previously reported[55]. A total of 511 (128 cases, 383 controls) of them who provided blood sample for telomere length measurement was included in the final analysis.

4.2.5 Statistical analysis

Baseline demographic and lifestyle information was compared by quintiles of TSR. Cox proportional hazard regression method was used to estimate hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) of developing gastric adenocarcinoma for different levels of quintile TSR for all subjects and by gender. Person-years of follow-up were computed from the blood sample collection date to the date of gastric cancer diagnosis, death, migration out of Singapore, or December 31, 2015, whichever occurred first. Age was calculated from the date of birth to blood sample collection date. To obtain the most recent information before blood collection, BMI, smoking status, pack-years of smoking, alcohol consumption status, and ethanol intake information were updated based on FU1 survey. If FU1 data was missing, then baseline information was used. The proportional hazards assumption was examined by testing the significance of Pearson's correlation coefficient between Schoenfeld residuals of the composite lifestyle score and ranked survival time[87]. We found no violation of proportional hazards

assumption. To characterize the dose-response associations between TSR level and risk of gastric adenocarcinoma, restricted cubic spline (RCS) analysis was applied and non-linearity test was conducted for the overall dose-response relation[102]. Predefined knots were located at 5th, 30th, 70th and 95th percentile of log transformed TSR distribution.

To adjust for potential confounding, age, sex, dialect group, year of recruitment, and level of education were included in all regression models. Further adjustment for other covariates such as intake of individual vegetables and fruit, family history of cancer and history of gastric/duodenal ulcer did not meaningfully alter the association between the composite lifestyle score and gastric adenocarcinoma risk. Thus the results presented were not adjusted for these variables.

Stratified analysis was conducted by gender and median age of gastric adenocarcinoma cases at 67 years old. To examine the potential modifying effect of subclinical symptoms of gastric cancer on the association between composite protective lifestyle score and gastric adenocarcinoma risk, we conducted sensitivity analyses on subset of dataset divided by the length of follow-up, e.g. ≤5 years and >5 years. To investigate the association between telomere length and gastric adenocarcinoma risk with adjustment of *H. pylori* infection status, conditional logistic regression model was performed in the nested case-control study for all subjects and for subjects with positive *H. pylori* infection status defined by positive CagA test results.

Dietary folacin, B6 and B12 intake were adjusted by total energy intake of per 1,000 kcal and quartile categorized. Based on ANOVA of average telomere length between each quartile, each nutrient was reclassified as dichotomized groups at different cutoffs (B6: 1st quartile; folate: 4th quartile; B12: median). Stratified analysis of telomere length and risk of gastric adenocarcinoma was conducted by each nutrient. Test for the interaction was conducted by

examining the statistical significance of interaction term between dichotomized nutrient intake and quintile telomere length in the Cox model.

All statistical analysis was performed in SAS 9.4 software package (SAS Institute, Inc.). All P values reported are two-sided. P values <0.05 were considered as statistically significant.

4.3 RESULTS

The comparison of baseline characteristics of 26,540 subjects by quintiles of TSR measurement was shown in **Table 3.1**. Overall, the mean of TSR was 1.02 with standard deviation of 0.23 (data not shown). Longer TSR measurement was characterized by higher age, females, higher education level (≥Secondary level), fewer current smokers and weekly drinkers, and higher sodium, vegetable and fruit intakes.

After 314,226 person-years of follow-up (mean 11.8 years per subject), as of December 31, 2015, a total of 265 incident cases of gastric cancer were identified. Among them, 17 cases were sarcoma, 12 lymphomas and 16 malignancies with unspecified histology; all of them were excluded from the present analysis. Thus the present study included 220 cases of gastric adenocarcinoma. The mean follow-up time from baseline to gastric adenocarcinoma diagnosis was 6.13 years (standard deviation=4.01).

Based on RCS analysis, a U-shaped association was found between TSR levels and risk of gastric adenocarcinoma (**Figure 2**) with a significant non-linear relation (*P*-value for non-linearity test=0.020). Extreme short length of telomere was statistical significantly associated with an increased risk of gastric adenocarcinoma, while there was an marginal significant association for extreme long telomere length (**Table 3.2**). Compared with the 2nd quintile, HRs (95% CI) for the lowest and highest quintile of TSR were 1.63 (1.08-2.47) and 1.55 (0.97-2.47), respectively after adjusting for age, sex, education, interview year, dialect group and smoking status. The U-shaped

association was stronger among males but no association was found among females (**Table 3.3**). The association was both present for short (≤5 years) and long (>5 years) duration of follow-up of the entire cohort (**Table 3.4**).

To examine whether *H. pylori* infection would impact the association between TSR levels and risk of gastric adenocarcinoma, a nested case-control study was conducted within the SCHS whose serological status of *H. pylori* infection was determined by the presence or absence of CagA in serum. After adjusting for CagA positivity status, the U-shaped association remained unchanged (**Table 3.5**). The association attenuated slightly after restricting to subjects with positive CagA status (**Table 3.5**).

4.4 DISCUSSION

In this prospective cohort study of 26,540 men and women aged 45 to 74, we found that extreme short and long telomere length was associated with an 63% and 55% increased risk of gastric adenocarcinoma. The association was even stronger among male participants. *H. pylori* infection status did not alter the observed association significantly.

Mounting evidence pointed out that short telomere length was associated with higher cancer risk including bladder, esophageal, gastric, head and neck, ovarian and renal[89]. The evidence on gastric cancer was solely based on retrospective studies. In a population-based case-control study conducted among residents aged between 21 to 79 years old in Poland, a total of 300 invasive gastric adenocarcinoma cases diagnosed between 1994 and 1996 and 416 matched controls were enrolled. Shortest quartile of telomere length almost doubled the risk of gastric cancer compared with the highest quartile (Odds Ratio=2.04, 95% CI: 1.33-3.13)[103]. In the same study, environmental factors such as positive *H. pylori* infection status, ever smoking and low fruit intake were found to have short telomere length[103]. Another hospital-based case-control study

conducted in China recruited 396 incident gastric adenocarcinoma cases diagnosed between 2007 and 2008 and 378 healthy controls. The below median telomere length was associated with increased risk of gastric adenocarcinoma (OR=2.14, 95% CI: 1.52-2.93)[44]. More recently, a community-based case-control study in China on 1,136 incident gastric cancer cases and 1,012 cancer-free controls showed a U-shaped association between telomere length and gastric cancer risk. Both the highest fifth quintile (OR=1.78, 95% CI: 1.30-2.44) and shortest first quintile (OR=3.81, 95% CI: 2.82-5.13) were associated with higher risk of gastric cancer compared with the fourth quintile[90]. Our findings were consistent with previous studies and further corroborate the association between extreme telomere length and high risk of gastric cancer in a prospective study setting.

There is inconsistent evidence regarding telomere length and gastric cancer survivability. A study based on two prospective cohorts of over 47,000 subjects among Danish general population found that shortening telomere length was not associated with risk of death (HR for 1 kilobase decrease in telomere length: 0.97, 95%CI: 0.57, 1.67). On the other hand, another study on 693 gastric cancer patients in China found an increased risk of mortality for short telomere length (HR=2.78, 95% CI: 1.24, 4.48) compared with long length. In our study, no association was found between telomere length and risk of mortality. However, there is a nonsignificant pattern of low survivability for both extreme long and short telomere length based on the adjusted survival curve. Our sample size of 155 deaths among 218 cases have only 75.9% power to detect a hazard ratio of 2.78. Future studies are needed to further clarify this association.

Emerged evidence could help explain the biological plausibility for the U-shaped association found in our study population. On one hand, shortened telomere length could lead to chromosome instability, cell inflammation and neoplastic changes in gastric mucosa cells during

early carcinogenesis. Previous study on 86 stage I-IV gastric cancer patients found that telomere length was significantly shorter in stage I tumor cells compared with its adjacent non-cancer mucosa[104]. In a matched case-control study using gastric mucosa biopsies from 217 gastric cancer patients and 102 controls, shortened telomere length was both associated with chronic inflammation (P=0.002) and intestinal metaplasia (P<0.001)[105]. Besides, short telomere length could induce epigenetic transformations. *In vitro* study of five gastric mucosa cell lines from cancer-free subjects, telomere shortening was associated with an 71% increased risk of hyper methylation in regional promoter CpG island[106].

On the other hand, later stage tumor cells were shown to have longer telomere length than their early stage counterparts[104]. One of the possible explanations was upregulation of telomerase, a reverse transcriptase enzyme helping maintain telomere length. Long telomere length thus increased chance of abnormality with occurrence of more cell divisions[48]. An increased activity of telomerase was reported in more than 85% of cancer cells[107]. In studies applying therapy to inhibit telomerase activity such as antisense human telomerase reverse transcriptase (ahTERT), decreased tumor cell proliferative and invasive capability and partial reverse of malignant phenotypes were found[108, 109]. Another explanation is that long telomere length in long term could intrigue overwhelming telomere maintenance mechanism and caused accelerated telomere length shortening, and thus breaking the homeostatic balance. In a human cell study, the researchers found a folate-deficient environment could not only induce long telomere length in short term and rapid telomere attrition, but also led to loss of terminal telomeric fragments[94]. In our study, we found that mean age and sex adjusted telomere length is 1.1% higher among highest quartile of dietary vitamin B12 intake compared with the lowest (p=0.025). Compared with lowest quintile of serum vitamin B12, subjects in the highest quintile also showed a 1.2% increased mean telomere length

(p=0.040) after controlling for age and sex. These results were consistent with a previous double-blind randomized clinical trial among subjects supplemented with vitamin B12, B6 and folic acid[97]. Vitamin B12 deficiency was associated with elevated methylmalonic acid and shortened telomere length[97]. Vitamin B12 is a critical player in one carbon metabolism as it is a coenzyme for methionine synthase to generate methionine from homocysteine[96, 97]. Vitamin B12 deficiency could results in homocysteine accumulation and lack of methionine, a methyl donor for the maintenance of DNA methylation and telomere length[92].

Genetic variants may also help elucidate the role of long telomere length in gastric cancer development. Evidence showed there was a low expression of genes controlling and decreasing telomere length. Compared with non-cancerous gastric mucosa tissue, a down-regulation of telomeric repeat binding factor 1 and 2 (TRF1, TRF2) and TRF1-interacting nuclear protein 2 (TIN2) mRNAs was detected in mucosa tissue from gastric cancer subjects[110]. Study also identified copy number changes in certain chromosome regions related with elevated telomerase activity level and longer telomere length among gastric cancer subjects[111].

We found a stronger association between telomere length and risk of gastric adenocarcinoma among male subjects but not among female subjects. One of the possible explanations is that worldwide males have higher background gastric cancer risk than females[18, 19]. The event rate in this study was higher among males (1,144.4 per 100,000) than among females (559.2 per 100,000). Relative risk difference due to variation of telomere length is more evident among higher absolute risk group. Also in our study females (TSR=1.05) have higher average telomere length than males (TSR=1.00). There are fewer female subjects in the shortest quintile of telomere length with higher risk of gastric adenocarcinoma.

The study association was only present among younger age group. Evidence regarding telomere length and gastric cancer risk by age groups is not consistent. While some study found the association between shortened telomere length and increase risk of gastric cancer in both younger and elder age group[44], other study found the association only among elder age group[103]. A case-control study on 598 cases and 2,212 controls found that the longest telomere length increased risk of colorectal cancer compared with the median among young age group (≤50 years), while shortest telomere length increased the risk among elder age group (>50 years)[112]. Our study finding of both extreme short and long telomere length in relation to increased risk of gastric adenocarcinoma only among the younger age group is inconsistent with previous ones. The underlying reason for a more pronounced association between telomere length and cancer risk in younger population is not clear. Telomere length on average shortens with the aging process. Extreme short telomere length among the younger group may indicate underlying variants on genes related with shortened telomere length. A meta-analysis of genome wide association studies (GWAS) on 21 cohorts of 48,000 individuals have identified multiple SNPs on candidate genes such as telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC) in relation to telomere length shortening[113]. On the other hand, extreme long telomere length among younger age group could possibly indicate a systematic disruption of telomere length maintenance and thus escape from programmed cell death. No association found among older age group could possibly be due to increased background risk of gastric cancer due to aging, and thus average out the telomere effect.

The strength of current study was its prospective study design, relative large sample size of more than 24,000 subjects with long-term follow-up of around 12 years, and serological status of *H. pylori* infection. We applied the monochrome multiplex quantitative PCR (MMQPCR) to

measure TSR. Compared with the conventional qPCR method, MMqPCR method reduced potential inter-sample error and normalizes differences in DNA concentration. There are several limitations in our study. All the information on lifestyle was self-reported at baseline with inherent non-differential misclassification. Also around 3% of subjects did not have follow-up survey information. Thus we could not obtain the updated information on smoking, alcohol drinking and BMI at time of blood collection. Our study population was middle-aged during baseline enrollment and thus we could do increased our study scope to younger population with average longer telomere length.

In conclusion, in this prospective cohort study we showed that either extreme short or long telomere length was associated with an increased risk of gastric adenocarcinoma among Chinese men and women in Singapore. Our study findings support the biological mechanism that both shortening and elongation of telomere length are involved in gastric carcinogenesis. Further prospective studies are needed to verify our findings in other populations.

APPENDIX A: MANUSCRIPT TABLES

A.1 TABLES FOR CHAPTER ONE

Table 1.1. Distribution of selected baseline characteristics of female participants of ever HRT and/or OC use, the Singapore Chinese Health Study, 1993-2013

Characteristics	Total cohort (n=34,022)	HRT[1] and/or OC use		*P* value[2]
		Ever (n=11,640)	Never (n=22,382)	
Age at interview (years), Mean (SD)	56.2 (8.0)	52.9 (6.2)	58.0 (8.3)	<0.001
Body mass index (kg/m^2), Mean (SD)	23.2 (3.3)	23.2 (3.3)	23.3 (3.3)	0.550
Total energy intake (Kcal), Mean (SD)	1,399.0 (472.6)	1,437.2 (468.7)	1,379.1 (473.5)	<0.001
Fruit intake (g/day), Mean (SD)	194.0 (162.1)	214.6 (168.2)	183.1 (157.7)	<0.001
Vegetable intake (g/day), Mean (SD)	109.9 (61.9)	116.4 (63.8)	106.5 (61.1)	<0.001
Sodium intake (mg/day), Mean (SD)	1010.7 (533.1)	1065.8 (536.8)	982.1 (529.0)	<0.001
Daily coffee drinker, N (%)	23,667 (69.6)	7,963 (68.4)	15,704 (70.2)	0.001
Education, ≥secondary level, N (%)	7,043 (20.7)	3,132 (26.9)	3,911 (17.5)	<0.001
Alcohol use, N (%)				<0.001
Nondrinker	30,933 (90.9)	10,379 (89.2)	20,554 (91.8)	
< 7 drinks/week	2,697 (7.9)	1,140 (9.8)	1,557 (7.0)	
≥7 drinks/week	392 (1.2)	121 (1.0)	271 (1.2)	
Smoking status, N (%)				<0.001
Never	31,058 (91.3)	10,954 (94.1)	20,104 (89.8)	
Former	841 (2.5)	202 (1.7)	639 (2.9)	
Current	2,123 (6.2)	484 (4.2)	1,639 (7.3)	
Age at menarche (years), Mean (SD)	14.4 (1.7)	14.1 (1.8)	14.6 (1.8)	<0.001
Age at menopause (years), Mean (SD)	49.4 (4.3)	48.9 (4.4)	49.6 (4.2)	<0.001
Nulliparous, N (%)	2,387 (7.0)	373 (3.2)	2,014 (9.0)	<0.001
# of live births among parous women, Mean (SD)	3.5 (1.6)	3.2 (1.4)	3.6 (1.6)	<0.001
Age at first birth, ≥31 years, N (%)	3376 (9.9)	956 (8.2)	2,420 (10.8)	<0.001
Menopausal status/type, N(%)				
Premenopausal	9,593 (28.2)	4,332 (37.2)	5,261 (23.5)	<0.001
Postmenopausal	24,429 (71.8)	7,308 (62.8)	17,121 (76.5)	
Natural	21,341 (87.4)	5,546 (75.9)	15,795 (92.3)	<0.001
Other means	3,088 (12.6)	1,762 (24.1)	1,326 (7.7)	

Table 1.1 Continued				
Hysterectomy/Oophorectomy status[3], N (%)				<0.001
No hysterectomy or oophorectomy	29,941 (89.1)	9,490 (82.3)	20,451 (91.9)	
Hysterectomy with no ovary removed	1,483 (4.4)	695(6.1)	788 (3.6)	
Hysterectomy+1 ovary removed	229 (0.7)	146 (1.3)	83 (0.4)	
Hysterectomy+2 ovaries removed	1,100 (3.3)	760 (6.6)	340 (1.5)	
Hysterectomy+ovaries removed (number unknown)	142 (0.4)	67 (0.6)	75 (0.3)	
Oophorectomy without hysterectomy	715 (2.1)	314 (2.7)	401 (1.8)	
HRT[1] and/or OC Use, N (%)				
HRT Only	----	2,651 (22.8)	----	
OC Only	----	7,348 (63.1)	----	
HRT and OC	----	1,641 (14.1)	----	

Table 1.2. Hazard ratios (HRs) and 95% confidence intervals (CIs) for reproductive factors and gastric cancer risk, The Singapore Chinese Health Study, 1993-2013

Characteristics[1]	Person-year	Cases (n)	HR (95% CI)[2]
Age at menarche			
<13 yrs	82,121	24	1.00 (ref.)
13-14 yrs	221,119	93	1.11 (0.70-1.74)
15-16 yrs	196,527	107	1.17 (0.74-1.86)
≥17 yrs	71,539	45	1.15 (0.69-1.93)
Parity			
Nulliparous	39,481	22	1.00 (ref.)
Parous	531,825	247	0.67 (0.43-1.04)
Number of Children			
0	39,481	22	1.00 (ref.)
1-2	164,171	56	0.69 (0.42-1.14)
3-4	217,593	76	0.59 (0.36-0.95)
≥5	150,061	15	0.74 (0.46-1.19)
Age at first birth among parous women			
≤20 yrs	106,249	76	1.00 (ref.)
21-25 yrs	219,053	97	0.80 (0.59-1.08)
26-30 yrs	148,292	55	0.87 (0.60-1.26)
≥31 yrs	57,859	19	0.76 (0.46-1.28)
Menstrual Status			
Premenopausal	170,727	34	1.00 (ref.)
Postmenopausal	400,579	235	1.29 (0.83-2.00)
Age at natural menopause			
<45 yrs	25,257	22	1.00 (ref.)
45-49 yrs	104,811	70	0.88 (0.54-1.42)
50-54 yrs	188,237	118	0.78 (0.49-1.23)
≥55 yrs	29,430	13	0.50 (0.25-0.99)[3]
Type of menopause			
Natural	347,734	223	1.00 (ref.)
Other means	52,845	12	0.48 (0.27-0.87)
Oophorectomy and hysterectomy status			
No Oophorectomy or hysterectomy	501,104	244	1.00 (ref.)
Hysterectomy with no ovary removed[4]	30,690	14	0.94 (0.55-1.61)
Oophorectomy with/without hysterectomy	37,949	11	0.68 (0.37-1.24)
Years of menstrual cycling[5]			
Quartile 1 (≤28.4)	122,299	75	1.00 (ref.)
Quartile 2 (>28.4 and ≤31.9)	139,923	51	0.67 (0.47-0.96)
Quartile 3 (>31.9 and ≤34.4)	136,550	82	0.91 (0.66-1.24)
Quartile 4 (>34.4)	119,048	49	0.67 (0.46-0.96)

[1] All the information is provided by the baseline interview only.

[2] Cox proportional hazard regression model included the following covariates: age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), body mass index (in kg/m^2), education (no formal/primary, ≥secondary education), smoking status (never/former, current), daily coffee drinking status (yes vs. no), and sodium intake (in mg/day).

[3] *P* for trend = 0.04.

[4] Includes women who had a hysterectomy, but did not know whether their ovaries were removed.

[5] Among premenopausal and natural postmenopausal women.

Table 1.3. Hazard ratios (HRs) and 95% confidence intervals (CIs) for exogenous hormone use and gastric cancer risk, The Singapore Chinese Health Study, 1993-2013

Characteristics	Person-years	Cases (n)	HR (95% CI)[1]
HRT use[2]			
Never	494,951	253	1.00 (ref.)
Ever	76,355	16	0.72 (0.43-1.21)
HRT use duration[2]			
Never users	498,227	253	1.00 (ref.)
<1yr	4,617	4	1.84 (0.68-4.95)
1-3 yrs	15,909	6	1.20 (0.53-2.73)
>3 yrs	52,553	6	0.40 (0.17-0.90)
OC use at baseline			
Never	415,452	228	1.00 (ref.)
Ever	155,854	41	0.67 (0.47-0.94)
Duration of OC use at baseline,			
Never users	415,452	228	1.00 (ref.)
≤ 2	75,879	19	0.66 (0.41-1.07)
>2	79,975	22	0.68 (0.43-1.05)[3]

Abbreviations: Hormone replacement therapy (HRT), Oral contraceptive (OC)

[1] Cox proportional hazard regression model included the following covariates: age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), body mass index (in kg/m^2), education (no formal/primary, ≥secondary education), smoking status (never/former, current), daily coffee drinking (yes, no), and sodium intake (in mg/day).

[2] HRT use is based on information provided at baseline and at follow-up interviews (see Methods for details).

[3] *P* for trend = 0.03.

Table 1.4. Odds rations(ORs) and 95% confidence intervals (CIs) of gastric cancer in relation to use of exogenous estrogen use, age at natural menopause and total years of menstrual cycling over lifetime among subjects with measurement of *H. pylori* infection (CagA) status, The Singapore Chinese Health Study

	All subjects		*H. pylori*-positive only	
	Ca/Co[1]	OR (95% CI)[2]	Ca/Co[1]	OR (95% CI)[2]
Total	48/139		48/115	
HRT[3] and/or OC use				
No	33/83	1.00 (ref.)	33/69	1.00 (ref.)
Yes	15/56	0.76 (0.33-1.76)	15/46	0.72 (0.30-1.71)
Years of menstrual cycling[4]				
Quartile 1 (≤28.4)	16/29	1.00 (ref.)	16/25	1.00 (ref.)
Quartile 2 (>28.4 and ≤31.9)	9/27	0.58 (0.19, 1.39)	9/24	0.54 (0.17, 1.67)
Quartile 3 (>31.9 and ≤34.4)	9/41	0.32 (0.10, 0.92)	9/32	0.35 (0.11, 1.11)
Quartile 4 (>34.4)	11/25	1.00 (0.34, 2.98)	11/18	1.20 (0.38, 3.78)

Abbreviations: Hormone replacement therapy (HRT), Oral contraceptive (OC)

[1] Number of cases (Ca) and controls (Co).

[2] ORs were derived from unconditional logistic regression models that included the following covariates: age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), body mass index (in kg/m^2), education (no formal/primary, ≥secondary), smoking status (never/former, current), daily coffee drinking (yes, no), sodium intake (in mg/day), and date of biospecimen collection.

[3] HRT use is based on information provided at baseline and at follow-up interviews (see Methods for details).

[4] Among premenopausal and natural postmenopausal women.

Table 1.5. Hazard ratios (HRs) and 95% confidence intervals (CIs) for exogenous hormone use and gastric cancer risk stratified by body mass index (BMI), The Singapore Chinese Health Study, 1993-2013

	BMI <median (23.2 kg/m^2)		BMI ≥median (23.2 kg/m^2)		*P* for interaction
Characteristics	Cases (n)	HR (95% CI)[1]	Cases (n)	HR (95% CI)[1]	
HRT use					
Never	110	1.00 (ref.)	143	1.00 (ref.)	0.8
Ever	8	0.72 (0.34-1.51)	8	0.72 (0.35-1.50)	
OC use at baseline					
Never	102	1.00 (ref.)	126	1.00 (ref.)	0.5
Ever	16	0.62 (0.36-1.06)	25	0.73 (0.46, 1.14)	
Duration of OC use at baseline, years					
Never users	102	1.00 (ref.)	126	1.00 (ref.)	0.8
≤ 2	6	0.48 (0.21-1.11)	13	0.85 (0.47-1.53)	
>2	10	0.74 (0.38-1.43)	12	0.62 (0.33-1.16)	

Abbreviations: Hormone replacement therapy (HRT), Oral contraceptive (OC)

[1] Cox proportional hazard regression model included the following covariates: age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), body mass index (in kg/m^2), education (no formal/primary, ≥secondary education), smoking status (never/former, current), daily coffee drinking (yes, no), and sodium intake (in mg/day).

[2] HRT use is based on information provided at baseline and at follow-up interviews (see Methods for details)

.

A.2 TABLES FOR CHAPTER TWO

Table 2.1. Distribution of selected baseline characteristics of all participants by composite lifestyle scores (5 factors), The Singapore Chinese Health Study, 1993-2014

Characteristics	Composite lifestyle scores			
	0/1/2	3	4	5
N	6,223	20,017	24,799	10,282
Age in years, mean (SD)	56.4 (7.7)	55.9 (7.9)	56.6 (8.1)	56.9 (7.9)
BMI (kg/m^2), mean (SD)	24.8 (4.5)	23.3 (3.5)	22.8 (2.8)	22.5 (2.4)
Female, %	21.8	50.5	61.5	71.2
Education level, %				
No formal education	20.2	24.7	30.0	30.0
Primary school	52.8	44.8	43.0	41.5
≥Secondary level	27.0	30.5	27.0	28.5
Smoking status, %				
Never smoker	25.4	63.5	78.2	87.0
Former smoker	21.3	12.8	8.6	6.3
Current smoker	53.3	23.7	13.2	6.7
Smoking amount among ever smokers, mean (SD)				
Number of cigarettes/day	24.1 (11.2)	18.1 (11.1)	12.3 (9.0)	9.2 (6.2)
Number of years of smoking	36.4 (8.8)	33.6 (11.4)	30.5 (12.7)	28.2 (13.6)
Pack-years of smoking	43.8 (21.9)	31.2 (22.4)	18.5 (16.4)	11.8 (7.4)
Alcohol consumption status, %				
Non-drinkers	58.9	77.9	86.4	91.4
Drinkers	41.1	22.1	13.6	8.6
Ethanol intake (g/day) among drinkers, mean (SD)	20.6 (22.3)	8.4 (14.2)	3.3 (5.9)	2.0 (1.8)
Daily sodium intake (mg), mean(SD)	1,607.9 (784.4)	1,361.0 (663.3)	915.1 (414.8)	816.9 (319.4)
Food consumption in grams, mean (SD)				
Total vegetables	113.5 (67.9)	110.2 (62.3)	99.2 (58.3)	137.3 (67.0)
Total fruits	183.9 (174.5)	194.7 (161.9)	191.1 (160.3)	256.8 (189.9)
Total red meat	46.9 (32.0)	37.7 (26.0)	25.6 (19.2)	18.7 (15.2)

Table 2.2. Lifestyle factors and gastric adenocarcinoma risk among all participants (n=61,321), The Singapore Chinese Health Study, 1993-2014

Characteristics	Person-years	All cases		Cardia		Non-cardia	
		Cases	HR (95% CI)[1]	Cases	HR (95% CI)[1]	Cases	HR (95% CI)[1]
Smoking							
Pack-years of smoking >21.9	132,949	187	1.00 (ref.)	37	1.00 (ref.)	128	1.00 (ref.)
Pack-years of smoking ≤21.9	906,112	504	0.66 (0.55-0.83)	81	0.54 (0.35-0.84)	363	0.66 (0.53-0.83)
Alcohol							
Daily ethanol intake >8.1g	62,238	70	1.00 (ref.)	11	1.00 (ref.)	51	1.00 (ref.)
Daily ethanol intake ≤8.1g	976,823	621	0.69 (0.53-0.89)	107	0.86 (0.45-1.63)	440	0.66 (0.49-0.89)
Body mass index (BMI)							
≥27.5 kg/m^2	89,879	66	1.00 (ref.)	16	1.00 (ref.)	47	1.00 (ref.)
<27.5 kg/m^2	949,182	635	0.80 (0.62-1.03)	102	0.54 (0.32-0.93)	444	0.79 (0.58-1.07)
Dietary pattern score							
<62	774,142	549	1.00 (ref.)	101	1.00 (ref.)	383	1.00 (ref.)
≥62	264,919	142	0.82 (0.68-0.99)	17	0.60 (0.35-1.01)	108	0.89 (0.71-1.11)
Sodium intake							
≥ 782 mg per 1,000 kcal energy	340,708	242	1.00 (ref.)	48	1.00 (ref.)	161	1.00 (ref.)
< 782 mg per 1,000 kcal energy	698,353	449	0.80 (0.68-0.94)	70	0.64 (0.44-0.93)	330	0.87 (0.72-1.05)
Composite scores (5 factors)[2]							
0/1/2	97,712	123	1.00 (ref.)	30	1.00 (ref.)	77	1.00 (ref.)
3	334,135	246	0.68 (0.52-0.88)	41	0.49 (0.30-0.79)	179	0.80 (0.61-1.04)
4	427,141	237	0.51 (0.40-0.66)	38	0.36 (0.22-0.60)	169	0.57 (0.43-0.76)
5	180,074	85	0.42 (0.31-0.57)	9	0.22 (0.10-0.47)	66	0.55 (0.39-0.78)
P trend			<0.001		<0.001		<0.001
PAR (95% CI)			0.48 (0.36-0.59)		0.72 (0.51-0.84)		0.43 (0.27-0.57)
Composite Z-score (5 factors)							
1st Quartile	251,028	212	1.00 (ref.)	44	1.00 (ref.)	140	1.00 (ref.)
2nd Quartile	258,747	167	0.76 (0.62-0.93)	29	0.64 (0.40-1.02)	129	0.88 (0.70-1.12)
3rd Quartile	263,404	162	0.72 (0.58-0.88)	25	0.54 (0.33-0.88)	109	0.72 (0.56-0.92)
4th Quartile	265,881	150	0.64 (0.51-0.78)	20	0.42 (0.25-0.71)	113	0.71 (0.55-0.91)
P trend			<0.001		0.001		0.002

Abbreviations: HR=hazard ratio; CI=confidence interval; PAR=population attributable risk

[1] For single lifestyle factor, model included all factors simultaneously and adjusted age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

[2] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal/primary, ≥secondary education).

Table 2.3. Composite lifestyle score and gastric cancer risk by gender and follow-up time, The Singapore Chinese Health Study, 1993-2014

Characteristics	N	Cases	Person-years	HR (95% CI)[1]
Male (N=27,293)				
Composite scores (5 factors)				
0/1/2	4,866	103	74,708	1.00 (ref.)
3	9,929	159	158,964	0.72 (0.56-0.92)
4	9,543	117	157,918	0.53 (0.40-0.69)
5	2,955	33	49,597	0.46 (0.31-0.69)
P trend				<0.001
PAR (95% CI)				0.50 (0.34-0.63)
Composite Z-scores (5 factors)				
1st Quartile	6,823	127	105,697	1.00 (ref.)
2nd Quartile	6,824	101	109,148	0.76 (0.58-0.99)
3rd Quartile	6,823	97	112,193	0.74 (0.56-0.96)
4th Quartile	6,823	87	114,148	0.62 (0.47-0.82)
P trend				0.001
Female (N=34,028)				
Composite scores (5 factors)				
0/1/2	1,357	19	23,004	1.00 (ref.)
3	10,088	88	175,171	0.67 (0.41-1.09)
4	15,256	120	269,223	0.52 (0.32-0.84)
5	7,327	52	130,477	0.47 (0.28-0.79)
P trend				0.003
PAR (95% CI)				0.41 (0.18-0.60)
Composite Z-scores (5 factors)				
1st Quartile	8,507	85	145,331	1.00 (ref.)
2nd Quartile	8,507	66	149,599	0.76 (0.55-1.05)
3rd Quartile	8,507	65	151,211	0.70 (0.50-0.96)
4th Quartile	8,507	63	151,733	0.66 (0.48-0.92)
P trend				0.011

Abbreviations: HR=hazard ratio; CI=confidence interval; PAR=population attributable risk

[1] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), and education (no formal education, primary education, ≥secondary education)

Table 2.4 Sensitivity analysis for composite lifestyle score and gastric adenocarcinoma risk by length of follow-up, The Singapore Chinese Health Study, 1993-2014

Follow-up time	Cases	Person-years	HR (95% CI)[1]
Follow-up ≤5 years			
Composite score (5 factors)			
0/1/2	36	30,149	1.00 (ref.)
3	68	97,487	0.71 (0.47-1.07)
4	51	121,335	0.44 (0.28-0.68)
5	15	50,499	0.33 (0.18-0.61)
P trend			<0.001
Composite Z-score (5 factors)			
1st Quartile	66	74,552	1.00 (ref.)
2nd Quartile	40	74,837	0.60 (0.41-0.89)
3rd Quartile	29	75,022	0.43 (0.28-0.66)
4th Quartile	35	75,058	0.50 (0.33-0.76)
P trend			<0.001
Follow-up >5 years			
Composite score (5 factors)			
0/1/2	86	67,563	1.00 (ref.)
3	178	236,648	0.71 (0.55-0.92)
4	187	305,805	0.57 (0.43-0.74)
5	70	129,575	0.52 (0.38-0.73)
P trend			<0.001
Composite Z-score (5 factors)			
1st Quartile	146	176,476	1.00 (ref.)
2nd Quartile	127	183,910	0.83 (0.66-1.06)
3rd Quartile	133	188,382	0.84 (0.66-1.07)
4th Quartile	115	190,823	0.70 (0.54-0.89)
P trend			0.007

Abbreviations: HR=hazard ratio; CI=confidence interval; PAR=population attributable risk

[1] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

Table 2.5. Composite lifestyle score and gastric adenocarcinoma risk among subjects with measurement of *H. pylori* infection (CagA) status, The Singapore Chinese Health Study, 1993-2014

Characteristics	All subjects			*H. pylori*-positive only[3]	
	Ca/Co	OR (95% CI)[1]	OR (95% CI)[2]	Ca/Co	OR (95% CI)[1]
All subjects	133/389			128/329	
Composite Score					
0/1/2	26/46	1.00 (ref.)	1.00 (ref.)	24/39	1.00 (ref.)
3	47/112	0.77 (0.43-1.40)	0.78 (0.43-1.42)	46/92	0.78 (0.42-1.46)
4	44/161	0.44 (0.24-0.81)	0.43 (0.23-0.81)	43/139	0.42 (0.21-0.82)
5	16/70	0.37 (0.16-0.82)	0.36 (0.16-0.82)	15/59	0.34 (0.15-0.80)
P trend		0.017	0.017		0.019
PAR (95% CI)		0.62 (0.26, 0.82)	0.62 (0.27, 0.83)		0.64 (0.28, 0.84)
Composite Z-score					
1st Quartile	48/110	1.00 (ref.)	1.00 (ref.)	46/94	1.00 (ref.)
2nd Quartile	28/89	0.71 (0.41-1.22)	0.68 (0.40-1.19)	28/76	0.70 (0.39-1.23)
3rd Quartile	34/90	0.79 (0.46-1.38)	0.80 (0.46-1.41)	32/77	0.75 (0.42-1.35)
4th Quartile	23/100	0.53 (0.28-0.98)	0.56 (0.29-1.06)	22/82	0.54 (0.27-1.05)
P trend		0.068	0.117		0.094
Cardia cases	24/69			21/60	
Composite Score					
0/1/2	6/8	1.00 (ref.)	1.00 (ref.)	5/8	1.00 (ref.)
3	7/22	0.46 (0.09-2.42)	0.45 (0.08-2.41)	7/17	0.58 (0.10-3.29)
4	9/27	0.28 (0.06-1.30)	0.28 (0.06-1.33)	8/25	0.33 (0.06-1.68)
5	2/12	0.20 (0.02-1.63)	0.20 (0.02-1.61)	1/10	0.11 (0.01-1.57)
P trend		0.074	0.075		0.065
Composite Z-score					
1st Quartile	11/23	1.00 (ref.)	1.00 (ref.)	10/20	1.00 (ref.)
2nd Quartile	4/15	0.47 (0.12-1.84)	0.47 (0.12-1.87)	4/13	0.53 (0.13-2.17)
3rd Quartile	5/17	0.29 (0.06-1.43)	0.29 (0.06-1.44)	4/14	0.33 (0.07-1.65)
4th Quartile	4/14	0.62 (0.15-2.59)	0.62 (0.15-2.59)	3/13	0.42 (0.08-2.24)
P trend		0.296	0.297		0.195
Non-cardia cases	88/253			87/212	
Composite Score					
0/1/2	17/27	1.00 (ref.)	1.00 (ref.)	16/23	1.00 (ref.)
3	34/74	0.69 (0.33-1.44)	0.73 (0.34-1.57)	34/60	0.76 (0.35-1.67)
4	27/106	0.31 (0.14-0.70)	0.34 (0.14-0.79)	27/90	0.35 (0.15-0.83)
5	10/46	0.24 (0.08-0.70)	0.28 (0.10-0.81)	10/39	0.29 (0.10-0.85)
P trend		0.009	0.023		0.026
Composite Z-score					
1st Quartile	31/68	1.00 (ref.)	1.00 (ref.)	30/59	1.00 (ref.)
2nd Quartile	20/58	0.72 (0.37-1.39)	0.72 (0.36-1.43)	20/49	0.72 (0.36-1.44)
3rd Quartile	24/59	0.76 (0.38-1.51)	0.86 (0.42-1.75)	24/50	0.87 (0.42-1.80)
4th Quartile	13/68	0.36 (0.15-0.83)	0.44 (0.19-1.05)	13/54	0.45 (0.19-1.08)
P trend		0.030	0.121		0.139

Abbreviations: Ca=Cases; Co=Controls; OR=odds ratio; CI=confidence interval, PAR=population attributable risk

[1] Conditional logistic regression model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

[2] Further adjusted for serum *H. pylori* CagA status (positive, negative).

[3] *H. pylori* positive defined by positive serum CagA status

Table 2.6. Selected food groups intake by high vs. low risk dietary pattern score, The Singapore Chinese Health Study, 1993-2014 (N=61,321)

Selected food groups	High risk (dietary pattern score<62)	Low risk (dietary pattern score≥62)	*P* value*
Total vegetables in g/day, mean (SD)	103.2 (58.7)	154.6 (72.3)	<0.001
Total fruits in g/day, mean (SD)	191.1 (161.2)	269.5 (198.3)	<0.001
Total red meat in g/day, mean (SD)	32.7 (24.9)	18.0 (15.1)	<0.001
Tofu products and soy drink in g/day, mean (SD)	106.9 (89.7)	141.0 (111.8)	<0.001
Rice in g/day, mean (SD)	423.3 (205.7)	376.9 (180.0)	<0.001
Noodles in g/day, mean (SD)	57.5 (47.7)	37.3 (36.9)	<0.001
Desert in g/day, mean (SD)	1.9 (4.3)	0.8 (2.3)	<0.001

* *P* values were derived from the Wilcoxon two-sample test.

Table 2.7. Scoring categorization of single lifestyle factor, The Singapore Chinese Health Study, 1993-2014 (N=61,321)

Lifestyle factor	Score (high-risk=0, low-risk=1)	
	High risk (%)	Low risk (%)
Cigarette smoking (pack-years of smoking)	>21.9 (14.7%)	≤21.9 (85.3%)
Daily ethanol intake (g)	>8.1 (6.3%)	≤8.1 (93.7%)
BMI (kg/m2)	≥27.5 (8.7%)	<27.5 (91.3%)
Sodium intake (g/per 1,000 kcal energy)	<782 (33.3%)	≥ 782(66.7%)
Dietary pattern score	<62 (25.2%)	≥62 (74.8%)

Table 2.8. Distribution of single lifestyle factor by composite lifestyle Z-score, The Singapore Chinese Health Study, 1993-2014 (N=61,321)

Characteristics	Composite lifestyle Z-scores			
	1st Qrt.	2nd Qrt.	3rd Qrt.	4th Qrt.
N	15,330	15,331	15,330	15,330
Pack-years of smoking, mean (SD)	20.2 (26.7)	9.4 (16.5)	4.8 (11.3)	1.6 (6.1)
Daily ethanol intake in grams, mean (SD)	4.8 (14.3)	1.4 (4.9)	0.7 (3.1)	0.3 (1.6)
Body mass index (BMI) in kg/m2, mean (SD)	24.9 (3.9)	23.4 (2.8)	22.7 (2.6)	21.5 (2.6)
Dietary pattern score, mean (SD)	35.9 (15.1)	45.3 (14.3)	52.9 (14.4)	65.5 (14.9)
Daily sodium intake, mean (SD)	855.7 (216.4)	743.7 (173.4)	658.2 (160.1)	543.2 (151.4)

Table 2.9. Distribution of single lifestyle factor by composite lifestyle score, The Singapore Chinese Health Study, 1993-2014 (N=61,321)

Characteristics	Composite lifestyle scores			
	0/1/2	3	4	5
N	6,223	20,017	24,799	10,282
Cigarette smoking, %				
Pack-years of smoking >21.9	66.0	19.8	3.6	0
Pack-years of smoking ≤21.9	34.0	80.2	96.4	100.0
Alcohol consumption, %				
Daily ethanol intake >8.1 g	35.8	6.8	1.0	0
Daily ethanol intake ≤8.1 g	64.2	93.2	99.0	100.0
Body mass index (BMI), %				
BMI ≥27.5 kg/m2	34.4	10.9	3.9	0
BMI <27.5 kg/m2	65.6	89.1	96.1	100.0
Dietary pattern score, %				
<62	99.4	96.5	81.9	0.0
≥62	0.6	3.5	18.1	100.0
Daily sodium intake, %				
≥782 mg/per 1,000 kcal energy	76.3	66.0	9.6	0
<782 mg/per 1,000 kcal energy	23.7	34.0	91.4	100.0

Table 2.10. Individual lifestyle factors and gastric adenocarcinoma risk, The Singapore Chinese Health Study, 1993-2014

Characteristics	Cases	Person-years	Base model[1] HR (95% CI)1	Mutually adjusted model[2] HR (95% CI)1
Pack-years of smoking				
Never smoker	382	749,840	1.00 (ref.)	1.00 (ref.)
Ever smoker				
1st quartile (>0 and ≤11.9)	57	78,593	1.02 (0.77-1.36)	1.02 (0.77-1.36)
2nd quartile (>11.9 and ≤21.9)	65	77,679	1.05 (0.79-1.38)	1.04 (0.79-1.38)
3rd quartile (>21.9 and ≤39.4)	122	82,660	1.62 (1.28-2.04)	1.58 (1.25-2.00)
4th quartile (>39.4)	65	50,289	1.40 (1.05-1.86)	1.33 (0.99-1.78)
P trend			<0.001	0.002
Daily ethanol consumption				
Non drinker	539	841,575	1.00 (ref.)	1.00 (ref.)
Ever drinker				
≤ median (8.1 g)	82	135,248	0.93 (0.74-1.18)	0.91 (0.72-1.16)
> median (8.1 g)	70	62,238	1.48 (1.14-1.91)	1.40 (1.08-1.83)
P trend			0.027	0.019
Body mass index (BMI)				
≤20 kg/m2	87	155,249	1.00 (ref.)	1.00 (ref.)
>20 and ≤24 kg/m2	383	561,651	1.16 (0.92-1.46)	1.19 (0.94-1.51)
>24 and ≤27.5 kg/m2	162	247,602	1.19 (0.91-1.54)	1.24 (0.96-1.61)
>27.5 kg/m2	59	74,459	1.53 (1.10-2.13)	1.58 (1.14-2.21)
P trend			0.025	0.057
Dietary pattern score				
1st quartile (≥1 and ≤37.5)	183	250,770	1.00 (ref.)	1.00 (ref.)
2nd quartile (>37.5 and ≤49.5)	164	255,550	0.89 (0.72-1.10)	0.94 (0.76-1.16)
3rd quartile (>49.5 and ≤62.0)	202	267,823	1.02 (0.84-1.26)	1.12 (0.91-1.38)
4th quartile (>62.0)	142	264,919	0.76 (0.61-0.95)	0.86 (0.68-1.09)
P trend			0.074	0.096
Sodium intake, mg/1000 kcal energy				
1st tertile (≤599)	235	341,474	1.00 (ref.)	1.00 (ref.)
2nd tertile (>599 and ≤782)	216	356,238	1.00 (0.83-1.21)	1.00 (0.83-1.21)
3rd tertile (>782)	240	341,349	1.27 (1.06-1.53)	1.25 (1.04-1.51)
P trend			0.010	0.024

[1] Based model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

[2] Mutually adjusted model included all the covariates in model A and mutually adjusted for all included lifestyle factors

Table 2.11. Z-score of individual lifestyle factors and gastric adenocarcinoma risk, The Singapore Chinese Health Study, 1993-2014

Z-score of each lifestyle factor	HR (95% CI)[1]	*P* value
Pack-years of smoking	1.09 (1.02-1.15)	0.005
Daily ethanol consumption	1.15 (1.05-1.26)	0.003
Body mass index (BMI)	1.09 (1.01-1.17)	0.028
Dietary pattern score	0.97 (0.90-1.06)	0.523
Daily sodium consumption	1.09 (1.01-1.18)	0.025

[1] For single lifestyle factor, model included all factors simultaneously and adjusted age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

A.3 TABLES FOR CHAPTER THREE

Table 3.1. Distribution of selected baseline characteristics of all participants by TSR, The Singapore Chinese Health Study, 1993-2015

Characteristics	TSR measurements				
	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile
N	5,038	5,038	5,038	5,038	5,038
Age in years, mean (SD)	65.9 (7.8)	63.8 (7.6)	62.7 (7.5)	61.5 (7.2)	60.1 (6.9)
BMI (kg/m2), mean (SD)	23.1 (3.5)	23.2 (3.5)	23.3 (3.5)	23.4 (3.5)	23.3 (3.5)
Female, %	44.8	50.2	53.2	59.1	62.2
Education level, %					
No formal education	22.5	21.1	20.5	20.6	19.4
Primary school	47.6	44.6	45.4	45.0	44.1
≥Secondary level	29.9	34.3	34.2	34.4	36.5
Smoking status, %					
Never smoker	60.5	65.4	68.3	72.0	74.4
Former smoker	21.6	17.4	15.3	13.5	12.4
Current smoker	17.9	17.2	16.4	14.4	13.2
Pack-years smoking among ever smokers, mean (SD)	33.6 (29.1)	30.8 (28.6)	30.5 (27.5)	28.1 (24.9)	27.9 (25.9)
Alcohol consumption status, %					
Non-drinkers	87.9	88.5	88.5	89.1	89.0
<7 drinks/week	8.4	8.4	8.5	8.4	8.4
≥7 drinks/week	3.7	3.1	3.0	2.5	2.6
Ethanol intake (g/day) among drinkers, mean (SD)	10.6 (16.5)	9.2 (15.2)	10.2 (16.9)	7.9 (13.6)	8.6 (15.0)
Daily sodium intake (mg), mean(SD)	1,122.5 (583)	1,136.9 (599.5)	1,149.7 (595.8)	1,142.3 (596.5)	1,162.8 (618.3)
Food consumption in grams, mean (SD)					
Total vegetables	112.2 (63.9)	115.9 (64.5)	116.3 (64.7)	116.7 (64)	119.6 (65.9)
Total fruits	205.6 (165.3)	216.4 (171.7)	210.3 (166.1)	214.9 (167.4)	221.4 (172.9)
Total red meat	30.7 (23.5)	31.1 (24.5)	31.7 (25.1)	31.2 (24.3)	31.4 (24.6)
Physical activity (hours/week), mean (SD)	5.0 (6.2)	4.7 (6.0)	4.9 (6.3)	4.7 (6.0)	4.7 (6.3)

Table 3.2. TSR decile and gastric cancer risk, The Singapore Chinese Health Study, 1993-2015

TSR level	Cases	Person-years	HR (95% CI)[1]
1st Quintile	65	59,791	1.63 (1.08-2.47)
2nd Quintile	34	62,053	1.00 (ref.)
3rd Quintile	41	63,024	1.30 (0.82-2.04)
4th Quintile	41	64,003	1.45 (0.92-2.29)
5th Quintile	39	65,355	1.55 (0.97-2.47)
$P_{\text{non-linear}}$[2]			0.020

Abbreviations: HR=hazard ratio; CI=confidence interval

[1] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, education (no formal education, primary education, ≥secondary education), and smoking status (never smoker, former smoker, current smoker)

[2] Based on RCS non-linearity test

Table 3.3. TSR decile and gastric cancer risk by gender, The Singapore Chinese Health Study, 1993-2015

TSR level	Male (n=12,234)			Female (n=14,306)		
	Cases	Person-years	HR (95% CI)[1]	Cases	Person-years	HR (95% CI)[1]
1st Quintile	47	31,406	1.75 (1.04-2.93)	18	28,385	1.42 (0.69-2.89)
2nd Quintile	21	29,425	1.00 (ref.)	13	32,627	1.00 (ref.)
3rd Quintile	25	27,903	1.33 (0.74-2.38)	16	35,121	1.24 (0.60-2.57)
4th Quintile	23	25,261	1.49 (0.82-2.70)	18	38,742	1.36 (0.66-2.78)
5th Quintile	24	23,602	1.88 (1.04-3.40)	15	41,753	1.15 (0.54-2.44)
$P_{\text{non-linear}}$[2]			0.009			0.782

Abbreviations: HR=hazard ratio; CI=confidence interval

[1] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, education (no formal education, primary education, ≥secondary education), and smoking status (never smoker, former smoker, current smoker)

[2] Based on RCS non-linearity test

Table 3.4. TSR decile and gastric cancer risk by duration of follow-up, The Singapore Chinese Health Study, 1993-2015

TSR level	Follow-up <5 years			Follow-up ≥5 years		
	Cases	Person-years	HR (95% CI)[1]	Cases	Person-years	HR (95% CI)[1]
1st Quintile	33	25,002	1.73 (0.95-3.15)	32	34,494	1.54 (0.86-2.75)
2nd Quintile	16	25,404	1.00 (ref.)	18	36,314	1.00 (ref.)
3rd Quintile	19	25,482	1.31 (0.67-2.55)	22	37,211	1.28 (0.69-2.40)
4th Quintile	15	25,566	1.18 (0.58-2.40)	26	38,084	1.66 (0.91-3.04)
5th Quintile	15	25,611	1.37 (0.67-2.79)	24	39,362	1.69 (0.91-3.15)
$P_{\text{non-linear}}$[2]			0.004			0.034

Abbreviations: HR=hazard ratio; CI=confidence interval

[1] Model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, education (no formal education, primary education, ≥secondary education), and smoking status (never smoker, former smoker, current smoker)

[2] Based on RCS non-linearity test

Table 3.5. TSR level and gastric adenocarcinoma risk among subjects with measurement of *H. pylori* infection (CagA) status, The Singapore Chinese Health Study, 1993-2015

TSR level	All subjects			H. pylori-positive only[3]	
	Ca/Co	OR (95% CI)[1]	OR (95% CI)[2]	Ca/Co	OR (95% CI)[1]
1st Quintile	37/98	2.00 (1.04-3.83)	1.94 (0.99-3.78)	37/85	1.92 (0.97-3.82)
2nd Quintile	18/87	1.00 (ref.)	1.00 (ref.)	18/74	1.00 (ref.)
3rd Quintile	29/78	2.04 (1.05-3.96)	2.04 (1.04-4.00)	29/64	2.02 (1.00-4.06)
4th Quintile	24/62	2.12 (1.05-4.30)	2.19 (1.05-4.54)	22/51	1.91 (0.89-4.12)
5th Quintile	20/58	2.14 (0.99-4.64)	2.20 (1.00-4.85)	17/50	1.64 (0.72-3.74)
$P_{\text{non-linear}}$[4]		0.038	0.031		0.073

Abbreviations: Ca=Cases; Co=Controls; OR=odds ratio; CI=confidence interval

[1] Conditional logistic regression model adjusted for age at baseline interview (in years), baseline interview year (1993-1995, 1996-1998), father's dialect (Cantonese, Hokkien), gender, and education (no formal education, primary education, ≥secondary education)

[2] Further adjusted for serum *H. pylori* CagA status (positive, negative).

[3] *H. pylori* positive defined by positive serum CagA status

[4] Based on RCS non-linearity test

APPENDIX B: MANUSCRIPT FIGURES

B.1 FIGURE FOR INTRODUCTION

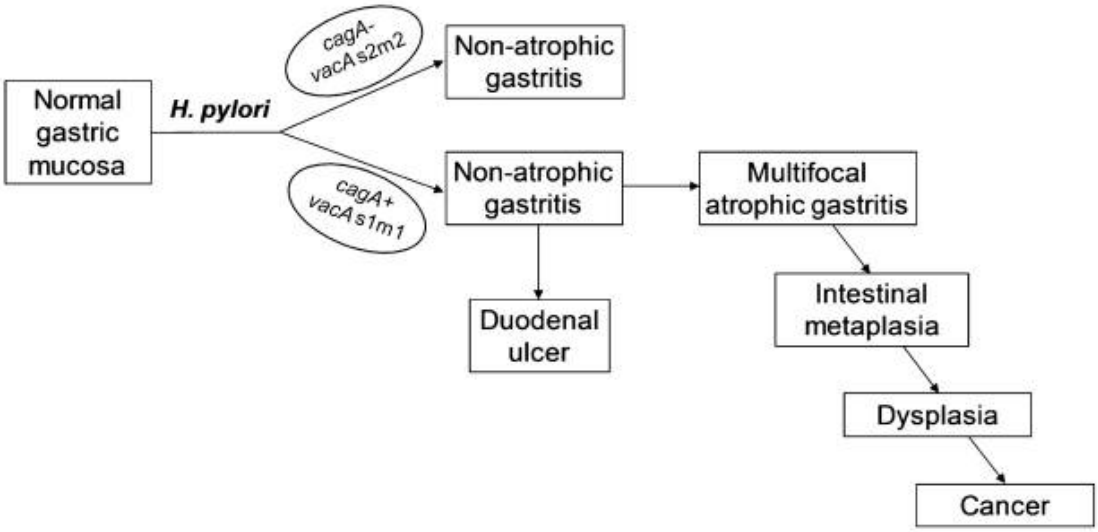

Figure 1. Correa's model for gastric carcinogenesis

B.2 FIGURE FOR CHAPTER ONE

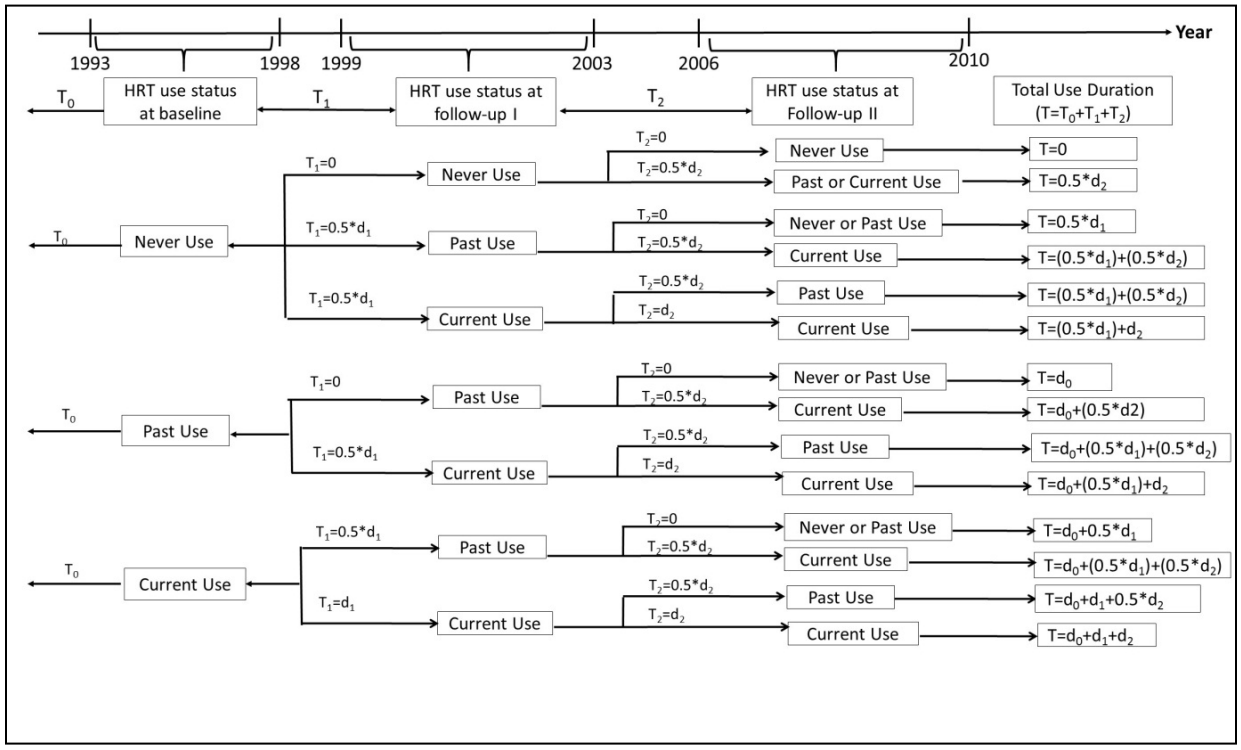

Figure 2. Flow diagram of computing hormone replacement therapy (HRT) use duration[1]

Abbreviations: FU1=follow-up I interview, FU2=follow-up II interview, d_0= HRT use duration reported until baseline, d_1= years between baseline and FU1 interview dates, d_2=years between FU1 and FU2 interview dates, T_0=self-report HRT use duration until baseline, T_1=computed HRT use duration between baseline and FU1 interview, T_2=computed HRT use duration between FU1 and FU2 interview.

[1] Total HRT use duration across the three time points (i.e., at baseline, baseline to FU1, and FU1 to FU2) was based on self-reported responses to never, past, or current HRT use at baseline, FU1 and/or FU2.

The followings are three examples to illustrate how total HRT use duration is computed:

1) If a woman reported never HRT use at baseline, past use at FU1 and current use at FU2, then total duration of HRT use would be calculated as follows:

0.5(years from baseline to FU1) + 0.5*(years from FU1 to FU2).*

Half of the years from baseline to FU1 and from FU1 to FU2 was included to estimate duration of use during these two periods, because the start date of HRT use was not obtained at FU1 or FU2.

2) If a woman reported past HRT use at baseline, and current use at FU1 and FU2, then total duration of HRT use would be calculated as follows:

(Duration reported at baseline) + 0.5(years from baseline to FU1) + (years from FU1 to FU2).*

3) If a woman report current HRT use at baseline, past use at FU1 and FU2, then total duration of HRT use would be calculated as follows:

(Duration reported at baseline) + 0.5(years from baseline to FU1)*

Since participants reported past HRT use at FU1 and FU2, we considered the past HRT use reported at FU2 as a repeat of past HRT use reported at FU1. Thus we assumed that the participant did not use HRT between FU1 and FU2.

B.3 FIGURE FOR CHAPTER THREE

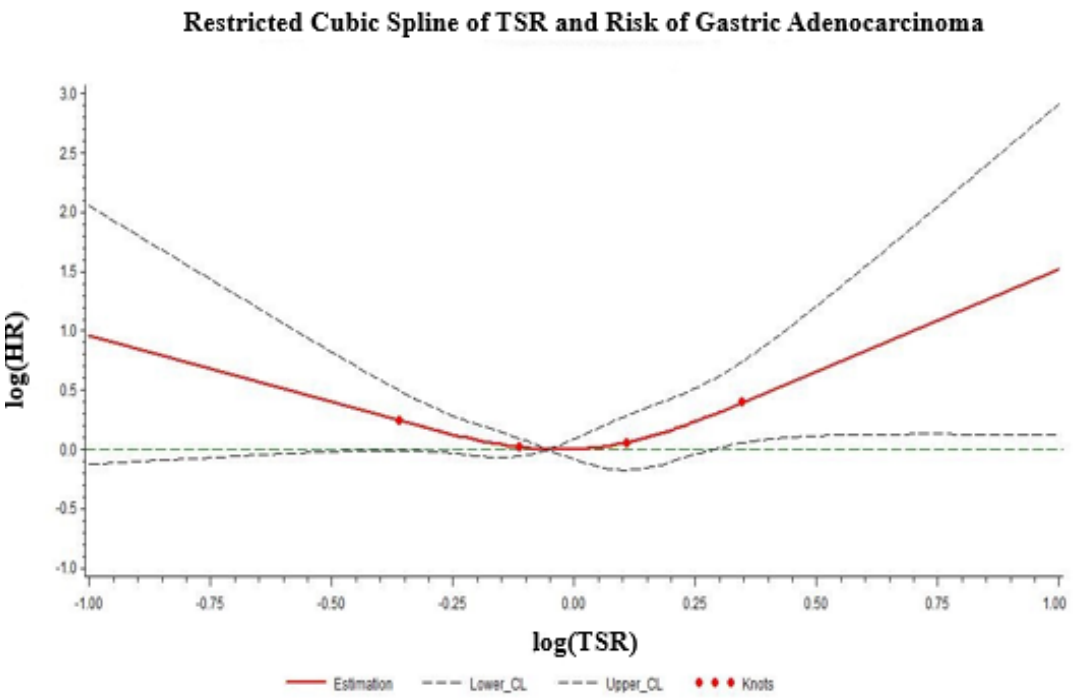

Figure 3. Restricted Cubic Spline[1] of TSR and Risk of Gastric Adenocarcinoma

Abbreviations: HR=hazard ratio; CI=confidence interval
[1] Restricted cubic spline at 4 knots located at 5th, 30th, 70th and 95th percentile of ln(TSR) distribution

BIBLIOGRAPHY

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on 02/14/2016.

2. Forman D, Bray F, Brewster DH, Gombe Mbalawa C, Kohler B, Piñeros M, Steliarova-Foucher E, Swaminathan R and Ferlay J, editors (2014) Cancer Incidence in Five Continents, Vol. X IARC Scientific Publication No. 164. Lyon: International Agency for Research on Cancer.

3. Singapore Cancer Registry Interim Report. Trends in Cancer incidence in Singapore 2010-2014. *Singapore: Ministry of Health* 2015; **Acessed Feb 17, 2016**.

4. Sankaranarayanan R., Swaminathan R., Lucas E. Cancer survival in Africa, Asia, the Caribbean and Central America (SurvCan). IARC Scientific Publications volume 162, ISBN 978-92-832-2162-3, Lyon, International Agency for Research on Cancer, 2011.

5. de Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012; **13**(6): 607-15.

6. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. H. pylori infection and gastric cancer: state of the art (review). *Int J Oncol* 2013; **42**(1): 5-18.

7. IARC Working Group: Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 61: 1-241, 1994.

8. Helicobacter, Cancer Collaborative G. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**(3): 347-53.

9. Conteduca V, Sansonno D, Ingravallo G, et al. Barrett's esophagus and esophageal cancer: an overview. *Int J Oncol* 2012; **41**(2): 414-24.

10. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans, volume 83. Tobacco Smoke and Involuntary Smoking. Lyon, France: IARC; 2004.

11. Ladeiras-Lopes R, Pereira AK, Nogueira A, et al. Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. *Cancer Causes Control* 2008; **19**(7): 689-701.

12. Miyoshi Y, Tanji Y, Taguchi T, Tamaki Y, Noguchi S. Association of serum estrone levels with estrogen receptor-positive breast cancer risk in postmenopausal Japanese women. *Clin Cancer Res* 2003; **9**(6): 2229-33.

13. Shin VY, Wu WK, Ye YN, et al. Nicotine promotes gastric tumor growth and neovascularization by activating extracellular signal-regulated kinase and cyclooxygenase-2. *Carcinogenesis* 2004; **25**(12): 2487-95.

14. Shin VY, Wu WK, Chu KM, et al. Functional role of beta-adrenergic receptors in the mitogenic action of nicotine on gastric cancer cells. *Toxicol Sci* 2007; **96**(1): 21-9.

15. Brenner H, Arndt V, Bode G, Stegmaier C, Ziegler H, Stumer T. Risk of gastric cancer among smokers infected with Helicobacter pylori. *Int J Cancer* 2002; **98**(3): 446-9.

16. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans, volume 96. Alcohol consumption and ethyl carbamate. Lyon, France: IARC; 2010.

17. Tramacere I, Negri E, Pelucchi C, et al. A meta-analysis on alcohol drinking and gastric cancer risk. *Ann Oncol* 2012; **23**(1): 28-36.

18. Zhang L, Eslick GD, Xia HH, Wu C, Phung N, Talley NJ. Relationship between alcohol consumption and active Helicobacter pylori infection. *Alcohol Alcohol* 2010; **45**(1): 89-94.

19. Zhu H, Yang X, Zhang C, et al. Red and processed meat intake is associated with higher gastric cancer risk: a meta-analysis of epidemiological observational studies. *PLoS One* 2013; **8**(8): e70955.

20. Jakszyn P, Bingham S, Pera G, et al. Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. *Carcinogenesis* 2006; **27**(7): 1497-501.

21. Jakszyn P, Agudo A, Lujan-Barroso L, et al. Dietary intake of heme iron and risk of gastric cancer in the European prospective investigation into cancer and nutrition study. *Int J Cancer* 2012; **130**(11): 2654-63.

22. Wang Q, Chen Y, Wang X, Gong G, Li G, Li C. Consumption of fruit, but not vegetables, may reduce risk of gastric cancer: results from a meta-analysis of cohort studies. *Eur J Cancer* 2014; **50**(8): 1498-509.

23. Mirvish SS. Inhibition by vitamins C and E of in vivo nitrosation and vitamin C occurrence in the stomach. *Eur J Cancer Prev* 1996; **5 Suppl 1**: 131-6.

24. Iijima K, Fyfe V, McColl KE. Studies of nitric oxide generation from salivary nitrite in human gastric juice. *Scand J Gastroenterol* 2003; **38**(3): 246-52.

25. Sorbye H, Svanes C, Stangeland L, Kvinnsland S, Svanes K. Epithelial restitution and cellular proliferation after gastric mucosal damage caused by hypertonic NaCl in rats. *Virchows Arch A Pathol Anat Histopathol* 1988; **413**(5): 445-55.

26. Furihata C, Ohta H, Katsuyama T. Cause and effect between concentration-dependent tissue damage and temporary cell proliferation in rat stomach mucosa by NaCl, a stomach tumor promoter. *Carcinogenesis* 1996; **17**(3): 401-6.

27. Wang XQ, Terry PD, Yan H. Review of salt consumption and stomach cancer risk: epidemiological and biological evidence. *World J Gastroenterol* 2009; **15**(18): 2204-13.

28. Murray L, Johnston B, Lane A, et al. Relationship between body mass and gastro-oesophageal reflux symptoms: The Bristol Helicobacter Project. *Int J Epidemiol* 2003; **32**(4): 645-50.

29. Yang P, Zhou Y, Chen B, et al. Overweight, obesity and gastric cancer risk: results from a meta-analysis of cohort studies. *Eur J Cancer* 2009; **45**(16): 2867-73.

30. Tu S, Bhagat G, Cui G, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 2008; **14**(5): 408-19.

31. Bianchini F, Kaaks R, Vainio H. Overweight, obesity, and cancer risk. *Lancet Oncol* 2002; **3**(9): 565-74.

32. Rotterdam H. Carcinoma of the stomach. New York Springer-Verlag, 2989.

33. de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. *Gastroenterol Clin North Am* 2013; **42**(2): 219-40.

34. Lauren P. Acta pathologica et microbiologica Scandinavica 1965;64:31-49.

35. Wu H, Rusiecki JA, Zhu K, Potter J, Devesa SS. Stomach carcinoma incidence patterns in the United States by histologic type and anatomic site. *Cancer Epidemiol Biomarkers Prev* 2009; **18**(7): 1945-52.

36. Correa P, Piazuelo MB. The gastric precancerous cascade. *J Dig Dis* 2012; **13**(1): 2-9.

37. Sipponen P, Correa P. Delayed rise in incidence of gastric cancer in females results in unique sex ratio (M/F) pattern: etiologic hypothesis. *Gastric Cancer* 2002; **5**(4): 213-9.

38. Dartois L, Fagherazzi G, Boutron-Ruault MC, Mesrine S, Clavel-Chapelon F. Association between five lifestyle habits and cancer risk: results from the E3N cohort. *Cancer Prev Res (Phila)* 2014; **7**(5): 516-25.

39. D'Elia L, Rossi G, Ippolito R, Cappuccio FP, Strazzullo P. Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies. *Clin Nutr* 2012; **31**(4): 489-98.

40. Abioye AI, Odesanya MO, Abioye AI, Ibrahim NA. Physical activity and risk of gastric cancer: a meta-analysis of observational studies. *Br J Sports Med* 2015; **49**(4): 224-9.

41. Lunet N, Valbuena C, Vieira AL, et al. Fruit and vegetable consumption and gastric cancer by location and histological type: case-control and meta-analysis. *Eur J Cancer Prev* 2007; **16**(4): 312-27.

42. Blackburn EH. Telomeres and telomerase: the means to the end (Nobel lecture). *Angew Chem Int Ed Engl* 2010; **49**(41): 7405-21.

43. Karlseder J. Telomere repeat binding factors: keeping the ends in check. *Cancer Lett* 2003; **194**(2): 189-97.

44. Liu X, Bao G, Huo T, Wang Z, He X, Dong G. Constitutive telomere length and gastric cancer risk: case-control analysis in Chinese Han population. *Cancer Sci* 2009; **100**(7): 1300-5.

45. Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005; **366**(9486): 662-4.

46. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002; **27**(7): 339-44.

47. Baird DM. Mechanisms of telomeric instability. *Cytogenet Genome Res* 2008; **122**(3-4): 308-14.

48. Cesare AJ, Reddel RR. Alternative lengthening of telomeres: models, mechanisms and implications. *Nat Rev Genet* 2010; **11**(5): 319-30.

49. Camargo MC, Goto Y, Zabaleta J, Morgan DR, Correa P, Rabkin CS. Sex hormones, hormonal interventions, and gastric cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2012; **21**(1): 20-38.

50. Persson C, Inoue M, Sasazuki S, et al. Female reproductive factors and the risk of gastric cancer in a large-scale population-based cohort study in Japan (JPHC study). *Eur J Cancer Prev* 2008; **17**(4): 345-53.

51. Freedman ND, Chow WH, Gao YT, et al. Menstrual and reproductive factors and gastric cancer risk in a large prospective study of women. *Gut* 2007; **56**(12): 1671-7.

52. Freedman ND, Lacey JV, Jr., Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. The association of menstrual and reproductive factors with upper gastrointestinal tract cancers in the NIH-AARP cohort. *Cancer* 2010; **116**(6): 1572-81.

53. Hankin JH, Stram DO, Arakawa K, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutr Cancer* 2001; **39**(2): 187-95.

54. Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice. *Cancer Res* 1999; **59**(19): 4823-8.

55. Ainslie-Waldman CE, Koh WP, Jin A, et al. Coffee intake and gastric cancer risk: the Singapore Chinese health study. *Cancer Epidemiol Biomarkers Prev* 2014; **23**(4): 638-47.

56. Veijola L, Oksanen A, Sipponen P, Rautelin H. Evaluation of a commercial immunoblot, Helicoblot 2.1, for diagnosis of Helicobacter pylori infection. *Clin Vaccine Immunol* 2008; **15**(11): 1705-10.

57. Duell EJ, Travier N, Lujan-Barroso L, et al. Menstrual and reproductive factors, exogenous hormone use, and gastric cancer risk in a cohort of women from the European Prospective Investigation Into Cancer and Nutrition. *Am J Epidemiol* 2010; **172**(12): 1384-93.

58. Sakauchi F, Japan Collaborative Cohort Study for Evaluation of C. Reproductive history and health screening for women and mortality in the Japan Collaborative Cohort Study for Evaluation of Cancer (JACC). *Asian Pac J Cancer Prev* 2007; **8 Suppl**: 129-34.

59. Frise S, Kreiger N, Gallinger S, Tomlinson G, Cotterchio M. Menstrual and reproductive risk factors and risk for gastric adenocarcinoma in women: findings from the canadian national enhanced cancer surveillance system. *Ann Epidemiol* 2006; **16**(12): 908-16.

60. Hemrika DJ, Slaats EH, Kennedy JC, de Vries Robles-Korsen TJ, Schoemaker J. Pulsatile luteinizing hormone patterns in long term oral contraceptive users. *J Clin Endocrinol Metab* 1993; **77**(2): 420-6.

61. Mishell DR, Jr., Kletzky OA, Brenner PF, Roy S, Nicoloff J. The effect of contraceptive steroids on hypothalamic-pituitary function. *Am J Obstet Gynecol* 1977; **128**(1): 60-74.

62. Kumtepe Y, Borekci B, Karaca M, Salman S, Alp HH, Suleyman H. Effect of acute and chronic administration of progesterone, estrogen, FSH and LH on oxidant and antioxidant parameters in rat gastric tissue. *Chem Biol Interact* 2009; **182**(1): 1-6.

63. Ames BN. Mutagenesis and carcinogenesis: endogenous and exogenous factors. *Environ Mol Mutagen* 1989; **14 Suppl 16**: 66-77.

64. Goldstein BD, Czerniecki B, Witz G. The role of free radicals in tumor promotion. *Environ Health Perspect* 1989; **81**: 55-7.

65. Farinati F, Cardin R, Degan P, et al. Oxidative DNA damage accumulation in gastric carcinogenesis. *Gut* 1998; **42**(3): 351-6.

66. Endogenous H, Breast Cancer Collaborative G, Key TJ, et al. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br J Cancer* 2011; **105**(5): 709-22.

67. Chavez-MacGregor M, van Gils CH, van der Schouw YT, Monninkhof E, van Noord PA, Peeters PH. Lifetime cumulative number of menstrual cycles and serum sex hormone levels in postmenopausal women. *Breast Cancer Res Treat* 2008; **108**(1): 101-12.

68. Ritte R, Tikk K, Lukanova A, et al. Reproductive factors and risk of hormone receptor positive and negative breast cancer: a cohort study. *BMC Cancer* 2013; **13**: 584.

69. Tom SE, Cooper R, Patel KV, Guralnik JM. Menopausal characteristics and physical functioning in older adulthood in the National Health and Nutrition Examination Survey III. *Menopause* 2012; **19**(3): 283-9.

70. McDowell MA, Brody DJ, Hughes JP. Has age at menarche changed? Results from the National Health and Nutrition Examination Survey (NHANES) 1999-2004. *J Adolesc Health* 2007; **40**(3): 227-31.

71. Heuch I, Kvale G. Menstrual and reproductive factors and risk of gastric cancer: a Norwegian cohort study. *Cancer Causes Control* 2000; **11**(9): 869-74.

72. Key TJ, Appleby PN, Reeves GK, et al. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br J Cancer* 2011; **105**(5): 709-22.

73. Motohashi M, Wakui S, Muto T, et al. Cyclin D1/cdk4, estrogen receptors alpha and beta, in N-methyl-N'-nitro-N-nitrosoguanidine-induced rat gastric carcinogenesis: immunohistochemical study. *J Toxicol Sci* 2011; **36**(3): 373-8.

74. Wakui S, Motohashi M, Muto T, et al. Sex-associated difference in estrogen receptor beta expression in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancers in rats. *Comp Med* 2011; **61**(5): 412-8.

75. Ohtani M, Ge Z, Garcia A, et al. 17 beta-estradiol suppresses Helicobacter pylori-induced gastric pathology in male hypergastrinemic INS-GAS mice. *Carcinogenesis* 2011; **32**(8): 1244-50.

76. Campbell-Thompson ML. Estrogen receptor alpha and beta expression in upper gastrointestinal tract with regulation of trefoil factor family 2 mRNA levels in ovariectomized rats. *Biochem Biophys Res Commun* 1997; **240**(2): 478-83.

77. Rio MC, Bellocq JP, Daniel JY, et al. Breast cancer-associated pS2 protein: synthesis and secretion by normal stomach mucosa. *Science* 1988; **241**(4866): 705-8.

78. Hanby AM, Poulsom R, Singh S, Elia G, Jeffery RE, Wright NA. Spasmolytic polypeptide is a major antral peptide: distribution of the trefoil peptides human spasmolytic polypeptide and pS2 in the stomach. *Gastroenterology* 1993; **105**(4): 1110-6.

79. Taupin D, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* 2003; **4**(9): 721-32.

80. Alison MR, Chinery R, Poulsom R, Ashwood P, Longcroft JM, Wright NA. Experimental ulceration leads to sequential expression of spasmolytic polypeptide, intestinal trefoil factor, epidermal growth factor and transforming growth factor alpha mRNAs in rat stomach. *J Pathol* 1995; **175**(4): 405-14.

81. Dunbier AK, Anderson H, Ghazoui Z, et al. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. *J Clin Oncol* 2010; **28**(7): 1161-7.

82. Yamachika T, Werther JL, Bodian C, et al. Intestinal trefoil factor: a marker of poor prognosis in gastric carcinoma. *Clin Cancer Res* 2002; **8**(5): 1092-9.

83. Xiao L, Liu YP, Xiao CX, Ren JL, Guleng B. Serum TFF3 may be a pharamcodynamic marker of responses to chemotherapy in gastrointestinal cancers. *BMC Clin Pathol* 2014; **14**: 26.

84. Buckland G, Travier N, Huerta JM, et al. Healthy lifestyle index and risk of gastric adenocarcinoma in the EPIC cohort study. *Int J Cancer* 2015; **137**(3): 598-606.

85. Butler LM, Koh WP, Lee HP, et al. Prospective study of dietary patterns and persistent cough with phlegm among Chinese Singaporeans. *Am J Respir Crit Care Med* 2006; **173**(3): 264-70.

86. World Health Organization. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; **363**(9403): 157-63.

87. Harrell F.E., K.L. L. Verifying Assumptions of the Cox Proportional Hazards Model. Proceedings of the Eleventh Annual. SAS User's Group International Conference. . Cary, NC: SAS Institute, Inc; 1986.

88. Spiegelman D, Hertzmark E, Wand HC. Point and interval estimates of partial population attributable risks in cohort studies: examples and software. *Cancer Causes Control* 2007; **18**(5): 571-9.

89. Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2011; **20**(6): 1238-50.

90. Du J, Zhu X, Xie C, et al. Telomere length, genetic variants and gastric cancer risk in a Chinese population. *Carcinogenesis* 2015; **36**(9): 963-70.

91. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst* 2013; **105**(7): 459-68.

92. Bull CF, O'Callaghan NJ, Mayrhofer G, Fenech MF. Telomere length in lymphocytes of older South Australian men may be inversely associated with plasma homocysteine. *Rejuvenation Res* 2009; **12**(5): 341-9.

93. Rane G, Koh WP, Kanchi MM, Wang R, Yuan JM, Wang X. Association Between Leukocyte Telomere Length and Plasma Homocysteine in a Singapore Chinese Population. *Rejuvenation Res* 2015; **18**(3): 203-10.

94. Bull CF, Mayrhofer G, O'Callaghan NJ, et al. Folate deficiency induces dysfunctional long and short telomeres; both states are associated with hypomethylation and DNA damage in human WIL2-NS cells. *Cancer Prev Res (Phila)* 2014; **7**(1): 128-38.

95. Mazidi M, Kengne AP, Banach M. Mineral and vitamin consumption and telomere length among adults in the United States. *Pol Arch Intern Med* 2017; **127**(2): 87-90.

96. Lee JY, Shin C, Baik I. Longitudinal associations between micronutrient consumption and leukocyte telomere length. *J Hum Nutr Diet* 2017; **30**(2): 236-43.

97. Pusceddu I, Herrmann M, Kirsch SH, et al. One-carbon metabolites and telomere length in a prospective and randomized study of B- and/or D-vitamin supplementation. *Eur J Nutr* 2016.

98. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241.

99. Forman D BF, Brewster DH, Gombe Mbalawa C, Kohler B, Piñeros M, Steliarova-Foucher E, Swaminathan R and Ferlay J, eds. Cancer Incidence in Five Continents, Vol. X IARC Scientific Publication No. 164. . Lyon, France: International Agency for Research on Cancer; 2014.

100. Zakian VA. Telomeres: the beginnings and ends of eukaryotic chromosomes. *Exp Cell Res* 2012; **318**(12): 1456-60.

101. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* 2009; **37**(3): e21.

102. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med* 2010; **29**(9): 1037-57.

103. Hou L, Savage SA, Blaser MJ, et al. Telomere length in peripheral leukocyte DNA and gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* 2009; **18**(11): 3103-9.

104. Mu Y, Zhang Q, Mei L, Liu X, Yang W, Yu J. Telomere shortening occurs early during gastrocarcinogenesis. *Med Oncol* 2012; **29**(2): 893-8.

105. Tahara T, Shibata T, Kawamura T, et al. Telomere length shortening in gastric mucosa is a field effect associated with increased risk of gastric cancer. *Virchows Arch* 2016; **469**(1): 19-24.

106. Tahara T, Shibata T, Okubo M, et al. Demonstration of potential link between Helicobacter pylori related promoter CpG island methylation and telomere shortening in human gastric mucosa. *Oncotarget* 2016; **7**(28): 43989-96.

107. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997; **33(**5): 787-91.

108. Yang SM, Fang DC, Yang JL, et al. Effect of antisense human telomerase RNA on malignant phenotypes of gastric carcinoma. *J Gastroenterol Hepatol* 2002; **17**(11): 1144-52.

109. Yang SM, Fang DC, Yang JL, Chen L, Luo YH, Liang GP. Antisense human telomerase reverse transcriptase could partially reverse malignant phenotypes of gastric carcinoma cell line in vitro. *Eur J Cancer Prev* 2008; **17**(3): 209-17.

110. Yamada M, Tsuji N, Nakamura M, et al. Down-regulation of TRF1, TRF2 and TIN2 genes is important to maintain telomeric DNA for gastric cancers. *Anticancer Res* 2002; **22**(6A): 3303-7.

111. Gumus-Akay G, Elhan AH, Unal AE, Demirkazik A, Sunguroglu A, Tukun A. Effects of genomic imbalances on telomerase activity in gastric cancer: clues to telomerase regulation. *Oncol Res* 2009; **17**(10): 455-62.

112. Boardman LA, Litzelman K, Seo S, et al. The association of telomere length with colorectal cancer differs by the age of cancer onset. *Clin Transl Gastroenterol* 2014; **5**: e52.

113. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 2013; **45(**4): 422-7, 7e1-2.