H. pylori Infection in Ontario: Prevalence, Risk Factors and Effect on the Bioavailability of Vitamins C and E

by

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Abstract

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*H. pylori* has been classified by World Health Organization as type I carcinogen for its association with gastric cancer. Among its suggested pathological pathways is oxidative stress, which may reduce the bioavailability of dietary antioxidants. The main objectives of this thesis were to estimate the prevalence of *H. pylori* infection in Ontario and to assess its effect on the bioavailability of two main dietary antioxidants, vitamins E and C.

To estimate the prevalence of *H. pylori* infection, a volume of 10 ul of plasma was aliquoted from stored blood of 1306 adults from Ontario. The blood samples belonged to controls of a population-based study of colorectal cancer. The overall weighted seroprevalence of *H. pylori* was 23.1% (95% CI: 17.7-29.5) with males having higher infection rates than females. Seroprevalence of the infection increased with age and number of siblings. Being non-white, born outside Canada and immigrating at an age greater than 20 years increased risk for *H. pylori* infection. An inverse association with seroprevalence was found for education and alcohol consumption.

In order to investigate whether *H. pylori* positive compared to *H. pylori* negative subjects have lower changes in plasma concentrations of ascorbic acid and alpha tocopherol when supplemented with these vitamins, *H. pylori* negative (n=32) and *H. pylori* positive (n=27) volunteers received vitamin C (500 mg) and alpha tocopherol (400 IU) supplementation daily for 28 days. *H. pylori* infection status was determined by $^{13}$C urea breath test. Post supplementation plasma ascorbic acid and alpha tocopherol were significantly higher than pre supplementation concentrations in both groups. The changes in plasma ascorbic acid and alpha tocopherol were not significantly different between *H. pylori*
negative and positive groups (ascorbic acid: 13.97±16.86 vs. 20.87±27.66, p=0.76; alpha
tocopherol: 15.52±9.4 vs. 14.47±15.77; p=0.39 for *H. pylori* negative and positive groups
respectively).

The weighted seroprevalence of *H. pylori* infection was 23.1%. Age, sex, ethnicity,
place of birth, age at immigration, education and alcohol consumption were factors
associated with the infection prevalence in the population studied. In addition, we found no
effect of *H. pylori* infection on the bioavailability of vitamins E and C.
To my mentor and uncle
Abdel Hamid Hallab,
Whose teachings were beyond words…
Acknowledgments

I would like to thank first and foremost God, my Creator and Sustainer, for all the mercies He has bestowed upon me and for answering my prayers to have the patience and strength to complete this work.

From the formative stages of this thesis, to the final draft, I owe an immense debt of gratitude to my supervisor, Dr. Nancy Kreiger. I thank her for appreciating my research strengths and patiently encouraging me to improve in my weaker areas. Her strong support of my ideas and research directions, and her confidence in my abilities motivated me to achieve beyond my own expectations. Graduate school can be a difficult, draining experience. I am proud to say my experience in her unit was intellectually exciting, and has energized me to plan for a career in academic research.

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Dissemination of research results


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<th>Description</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Acetaminophen</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index <em>H. pylori</em></td>
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<tr>
<td>CADET-PE</td>
<td>Canadian Adult Dyspepsia Empiric Treatment - Prompt Endoscopy</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CO2</td>
<td>Carbon Dioxide</td>
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<td>DHAA</td>
<td>Dehydroascorbic acid</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>ELISA</td>
<td>Enzyme-Linked Immuno absorbent Assay</td>
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<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
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<td>GERD</td>
<td>Gastro Esophageal Reflux Disease</td>
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<td><em>H. pylori</em></td>
<td><em>Helicobacter pylori</em></td>
</tr>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>HPLC</td>
<td>High performance Liquid Chromatography</td>
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<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
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<tr>
<td>IARC</td>
<td>International Agency for Cancer Research</td>
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<tr>
<td>Log</td>
<td>Logarithm</td>
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<td>MDA</td>
<td>Malondialdehyde</td>
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<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>Ns</td>
<td>Non significant</td>
</tr>
<tr>
<td>OFCCR</td>
<td>Ontario Familial Colon Cancer Registry</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive Nitrogen Species</td>
</tr>
<tr>
<td>Rpm</td>
<td>Round per minute</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>Resp.</td>
<td>Respectively</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SES</td>
<td>Socio Economic Status</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>UBT</td>
<td>Urea Breath Test</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>Yrs</td>
<td>Years</td>
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Chapter 1: Introduction

1.1 Rationale

*H. pylori* infection has been recognized as having a prominent causative role for gastric cancer; the latter being the fourth most common cancer and the second most common cause of cancer deaths worldwide (WCRF, 1997). Since its discovery in the 1980’s, *H. pylori* has been researched extensively in the epidemiology and microbiology fields. To date, given the established relationship between *H. pylori* and gastric cancer, the most important question in *H. pylori* research remains whether to test for and treat for this infection? The answer to this question lies in better understanding the still obscure areas in the epidemiology and pathology of this bacterium.

At the epidemiological level, this bacterium has been found to be the most common infectious disease in the world (Pounder et al., 1995). Its prevalence varies between developing and developed countries; prevalences among adults are typically 80-90% and <40% respectively. Among other risk factors, sex, age, social class and number of siblings have been consistently associated with this infection (Constanza et al., 2004).

In Canada, few studies estimated the prevalence of the infection and due to their significant limitations (i.e. their focus on high risk populations and/or their small sample size) their results can not be applied to the general Canadian population (Sullivan et al., 2004). Given the ongoing debate about the efficacy of *H. pylori* eradication, it is important and timely to obtain a prevalence estimate for this infection in Canada. In addition, investigation of the risk factors associated with this infection is crucial to identify high-risk groups in the population and consequently to develop appropriate public health intervention strategies.
Although understanding the epidemiology of the infection is important to define the scope and magnitude of its impact on the public health, parallel effort should be invested in understanding the pathogenesis of diseases associated with *H. pylori* infection. On one hand, it has been suggested that reactive oxygen species (ROS) production with damage to cell macromolecules, including DNA, may be an important mechanism of *H. pylori* action (Khanzode et al., 2003). On the other hand, diet has been linked with the etiology of gastric cancer in numerous international studies. Vitamins E and C intakes are postulated to protect against development of gastric cancer (Flora, 2007). A possible explanation of this protective effect is that these vitamins, being the main dietary antioxidants, are utilized in neutralizing the reactive oxygen species produced as a result of *H. pylori* infection (Boone et al., 1997). Therefore, it is important to study the possible interaction between *H. pylori* infection and vitamins C and E in order to better understand the complexity of the pathology of this bacterium.

### 1.2 Objectives

The studies presented in this dissertation aim at shedding the light on two main areas in *H. pylori* research: epidemiology and patho-physiologic basis of *H. pylori* associated diseases. Below is a description of the objectives of each project:

- **Project I: Prevalence of *H. pylori* infection in Ontario**
  
The main objective of this research is to estimate the prevalence of *H. pylori* infection in Ontario. In addition this project aims to identify risk factors associated with the infection in the Canadian population such as ethnicity, socioeconomic status and lifestyle habits.
• Project II: Effect of *H. pylori* infection on the bioavailability of vitamins C and E.

Project II hypothesizes that *H. pylori* infection, by increasing the production of reactive oxygen species and consequently the utilization of dietary antioxidants mainly vitamins E and C that serve in quenching free radicals, decreases the serum levels of these vitamins and reduces their protective effect against gastric cancer. The objective of this project is to assess the effect of *H. pylori* infection on the bioavailability of vitamins C and E.
Chapter 2: Literature review

2.1 *H. pylori* infection overview

Although it is generally believed that *H. pylori* is a recent discovery, this bacterium was first isolated and described in pathologic specimens in the 1890’s and again in the 1930’s (Go, 2002). In 1980, Marshall and Warren cultured *H. pylori*, proving its infectious nature, and described associated clinical symptoms (Marshall et al., 1984).

*H. pylori* is a spiral-shaped, gram negative, microaerophilic rod with 4-7 flagella. The flagella are essential for the motility of the bacterium and its ability to remain in the mucus layer of the stomach (Ottemann et al., 2002). Approximately 20% of *H. pylori* in the stomach are found adhered to the surfaces of the mucus epithelial cells (Hessey et al., 1990). *H. pylori* possesses over 30 genes dedicated to the expression of outer membrane proteins, many of which act as adhesins such as BabA, SabA, HopZ, HopH, AlpA and AlpB (Peck et al., 1999; Dossumbekova et al., 2006). In their review Amieva and his colleagues (2008) suggested that the main reasons for adhesion are (i) to cause cellular damage and inflammation, (ii) to avoid mechanical clearance, (iii) and to use the cell surface as a site for replication. Another essential factor in *H. pylori* colonization of the human stomach is the urease activity of this bacterium, which enables *H. pylori* to survive the acidic gastric environment (Weeks et al., 2000). In this section, a background of the epidemiology of *H. pylori*, diagnostic tests for the infection and the main treatment regimens will be presented in addition to its relation to various diseases including gastric cancer.
2.1.1 Sources and modes of transmission of *H. pylori* infection

Humans are the only known reservoir for *H. pylori* (Nakamura, 2001). While *H. pylori*’s main habitat is the stomach, some studies have reported the presence of this microorganism in saliva and dental plaque (Gebara et al., 2006; Hooshmand et al., 2004). Transmission is ‘opportunistic’ in that any method that allows the organism’s access to the stomach is likely to be a mode of transmission. The evidence overwhelmingly supports person-to-person transmission as the predominant mechanism, although few investigations have been able to discriminate between direct transmission from one person to another, and common sources of infection associated with crowding. Various routes of direct transmission have been described in the literature, mainly fecal-oral, oral-oral and gastric-oral routes. *H. pylori* has been cultured from vomitus, diarrhoeal stools and dental plaque, demonstrating that the bacterium is transmissible by these 3 routes (Graham et al., 1992).

As for indirect modes of transmission, transient or persistent survival of *H. pylori* in environmental reservoirs has been hypothesized to promote the spread of the infection. In areas with lax sanitation, contaminated water or food may play a role in transmission; however, the culture of *H. pylori* from any of these sources is very rare (Brown, 2000). It has been suggested that *H. pylori* may exist in the environment in a dormant, spore like state that can be viable but not culturable. This hypothesis stems from the fact that, under stressful conditions, *H. pylori* undergoes a morphologic transformation from actively dividing and swimming spiral bacilli to inactive cocci (Amieva et al., 2008). Whether *H. pylori* can revert from the coccoid form to the infectious spiral bacilli is still unknown.

In order to reduce the rate of transmission, an improvement in overall sanitation, including clean water, waste disposal, and household hygienic practices, is required.
(Moayyedi et al., 2002). Low socioeconomic status (SES) in addition to other risk factors for 
*H. pylori* infection will be discussed in the following section.

### 2.1.2 Risk Factors for *H. pylori* infection

Over the past 10 years, extensive study of *H. pylori* using various diagnostic 
techniques revealed certain demographic patterns, mainly higher prevalence of the infection 
with increasing age, in larger families, in certain ethnic groups and in males.

**Age**

Most cross sectional studies that investigated the relationship between *H. pylori* 
infection prevalence and age resulted in a positive association (Everhart et al., 2000; 
Constanza et al., 2004; Akin et al., 2004; Robertson et al., 2003; Koch et al., 2005). Whether 
this observed increase of infection prevalence with age is a result of a cohort effect or 
indicates that age is a risk factor for the infection is debatable. Banatvala et al., (1993) 
demonstrated that *H. pylori* infection is actually becoming less frequent and is predominantly 
acquired in childhood. A total of 631 serum samples were collected from adults and children 
and tested for *H. pylori* infection in 1969, 1979. 1989. By studying seropositivity by year of 
birth, the magnitude of a cohort effect of *H. pylori* seropositivity was estimated. The odds of 
being seropositive decreased by 26% per decade, $P = .008$ (95% CI: 8%-41%). The authors 
suggested that this effect is a by-product of the lower sanitary condition and less prevalent 
use of antibiotics in older cohorts. On the other hand, Veldhuyzen et al., (1994) showed that a 
continuous risk of acquisition of 1% per year rather than a cohort effect best explains the 
pattern of *H. pylori* infection in a cohort of 316 subjects followed over the period of 3 years.
**Number of siblings**

Increased number of siblings is among the established risk factors for *H. pylori* infection (Goodman et al., 2000). Some reports suggested that, given that the number of siblings and socioeconomic status are highly correlated, the effect of the former on the incidence of infection is confounded by socioeconomic variables such as education and income. In a study of *H. pylori* seroprevalence in adults from Ankara, Turkey, household size of 4 and above was positively associated with *H. pylori* infection prevalence even after adjustment for SES. Compared to individuals from households of size <4, those from households of 5–6 individuals, and households of at least 7 members were significantly more likely to have *H. pylori* infection: the ORs (95% CI) were 1.74 (1.56–1.93) and 1.36 (1.15–1.60) respectively (Akin et al., 2004). These finding were echoed in Brazil, where crowding was found to be a risk factor for the infection independent of income and education level (Zaterka et al., 2007). Whether this effect is due to sharing a common exposure source or transmission among individuals is not known.

**Ethnicity**

Differences in *H. pylori* prevalence among ethnic groups have been described in many studies assessing risk factors for the infection. In the United States, age adjusted prevalence was substantially higher among non-Hispanic blacks (52.7%) and Mexican Americans (61.6%) than among non Hispanic whites (26.2%) (Everhart et al., 2000). In Brazil, prevalence of *H. pylori* is higher in Non-White population, independent of gender (Zaterka et al., 2007).
**Sex**

Another suggested risk factor for *H. pylori* infection is the male sex. In a meta-analysis aimed at investigating the effect of sex on *H. pylori* infection, males had a higher rate of infection than females (OR=1.2, 95% CI: 1.02-1.4) (Replogle et al., 1995). Similar findings were reported by a more recent meta analysis of population based prevalence surveys which showed comparable rates of *H. pylori* infection among males and females during childhood (OR= 1.03, 95% CI: 0.91-1.17) and an elevated risk for males in adulthood (OR= 1.16, 95% CI: 1.11-1.22) (De Martel et al., 2006). Two possible explanations are differential immunocompetence and differential exposure. If relative immunodeficiency were operating, one would expect higher rates of infection among males during childhood as well as adulthood. Therefore, differential exposure or eradication may explain the differences between males and females. Physical contact increases the risk of exposure to the bacterium (Replogle et al., 1995). Continued participation in activities involving physical contact is generally accepted for males at all ages, while similar behaviour is often discouraged for females beyond childhood (Asaka et al., 2001). Furthermore, it is possible that women are more likely than men to have infection eradicated with antimicrobials used for other illnesses (Bytzer et al., 2005).

**Socioeconomic status (SES)**

Among environmental and lifestyle factors, low SES and smoking have been associated with an increased risk of *H. pylori* infection. Several studies worldwide have suggested a negative association between SES and *H. pylori* seroprevalence. Akin et al., (2004) showed that subjects with low SES had higher prevalence of the infection compared to moderate SES (males: OR: 2.42 CI: 2.11-2.77; females: OR: 1.77 CI: 1.55-2.03). Everhart
et al. (2000) had similar findings when they demonstrated that subjects at or higher than the poverty level had significantly less prevalence of the infection compared to those below that level (30.2% vs. 48% respectively).

**Smoking**

The association between *H. pylori* infection and smoking is controversial. In an investigation into the factors associated with *H. pylori* infection in Glasgow, U.K., Woodward et al. (2000) found that ex-smokers (71%) and current cigarette smokers (73%) have higher prevalences than never-smokers (59%), but similar prevalences to each other. After adjustment for age, social class, sex, and number of siblings, smokers and ex-smokers were at a higher risk for the infection than subjects who never smoked (OR: 1.5; 95%CI: 1.1-1.9 and OR: 1.4; 95%CI: 1.1-2 respectively). Other reports from the literature showed no association of smoking with *H. pylori* infection (Constanza et al., 2004; Akin et al: 2004; Everhart et al., 2000). Smokers often share cigarettes; therefore the act of smoking involves inserting orally the tip of a cigarette, which may well have been handled by other people (Tyas et al., 1998). However, smoking is also characteristic of low social status (Fiedorek et al., 1991; Al Moagel et al., 1990), and the smoking effect could simply be a proxy for other socioeconomic factors such as income and education.

**Other factors**

In addition to the aforementioned suggested risk factors for *H. pylori* infection, lower Body Mass Index (BMI), incidence of diabetes and colon adenomatous polyps, regular intake of aspirin, acetaminophen and lower fruits and vegetables intake and more coffee consumption have been cited in few reports to have a positive association with the infection (Everhart et al., 2000; Ioannou et al., 2005; Hunt et al., 2004; De Koster et al., 2000).
However the evidence for a relationship between these factors and the risk for *H. pylori* infection is not conclusive and further investigation in this field is warranted.

Better understanding of the sources and modes of transmission of *H. pylori* and studies of risk factors were made possible by the recent advances in the diagnostic tests for detection of infection with this bacterium. These tests will be described in detail in the following section.

### 2.1.3 Diagnosis and treatment of *H. pylori* infection

#### Diagnosis of *H. pylori* infection

The reference method for diagnosis of active *H. pylori* infection is esophago-gastro-duodenoscopy with gastric biopsies. At present there are numerous other accurate detection assays (Gisbert et al., 2005). These assays are classified as either invasive (e.g. gastric biopsy) or non-invasive (e.g. stool, blood or breath tests). The choice of the test depends of the clinical circumstances, the cost effectiveness and the availability of the test.

The most commonly used tests for diagnosis of *H. pylori* are serology, urea breath test (UBT), and culture of gastric biopsies. Serologic testing is comparatively less expensive than other methods, though less sensitive and specific (Lacy et al., 2001). The results of a Canadian economic analysis, comparing the cost effectiveness of empirical ranitidine with a test and treat strategy using either *H. pylori* serology or $^{13}$C urea breath test in low risk patients with uninvestigated dyspepsia, showed that testing for *H. pylori* infection by serology appeared more economical (Marshall et al., 2000). Table 2.1 summarizes the sensitivity and specificity of the different tests used in the diagnosis of *H. pylori* in adult populations.
Table 2.1 Sensitivity and specificity of available diagnostic tests for *H. pylori* infection

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity*</th>
<th>Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non invasive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serology (use of serum ELISA to check for IgG antibodies)</td>
<td>80%</td>
<td>90%</td>
</tr>
<tr>
<td>Urea breath test (based on the urease enzyme production by <em>H. pylori</em> organisms)</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Stool <em>H. pylori</em> antigen test</td>
<td>80%</td>
<td>94%</td>
</tr>
<tr>
<td><strong>Invasive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric biopsy:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological staining of gastric biopsies</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>Culture of gastric biopsy specimens</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Rapid urease test of a gastric biopsy</td>
<td>95%</td>
<td>98%</td>
</tr>
</tbody>
</table>

* Nakamura, (2001); Lacy, (2001)

Culture and tests based on the detection of *H. pylori* urease are suited to detect active infection, whereas all the other tests—especially serology tests—also allow the detection of infections that are no longer active (Hirschl et al., 2007). Culture from gastric biopsy is considered the gold standard. In some instances, the accuracy of culture test fluctuates due to sampling error at the time of the biopsy (because *H. pylori* may be distributed unevenly in the stomach), variations in the quality of the specimens and proper transportation conditions (Chiba, 1996; Hirschl et al., 2007). To overcome the sampling error, it is recommended that physicians take a minimum of 4 biopsy specimens (2 from the lesser curvature and 2 from the greater curvature sites) (Graham et al., 2008). For population applications, in the absence of a cost-effective study comparing UBT to serology in cancer screening, serology may be more appropriate for asymptomatic patients because of its relatively lower cost and acceptable sensitivity and specificity.

In children, the accuracy of the UBT is challenged by the difficulty to perform this test especially in very young children and the existence of other oral micro-organisms that
produce the urease enzyme (Jones et al., 2001). The stool antigen test is an accurate non-invasive diagnostic test in children and has the potential to be the method of choice for *H. pylori* diagnosis in pediatric populations (Ni et al., 2000). Further studies are warranted to validate the sensitivity and specificity of the stool antigen test in children.

**Treatment of *H. pylori* infection**

The Canadian guidelines for treatment of *H. pylori* infection, issued by the Canadian *H. pylori* Study Group (CHSG), recommend screening for and treating *H. pylori* infection in all patients with duodenal or gastric ulcers, whether symptomatic or asymptomatic. In addition, eradication treatment is recommended upon confirmation of *H. pylori* infection (Hunt et al., 1999).

The success of *H. pylori* infection treatment depends on two main factors: antibiotic resistance and compliance to the treatment regimen, the latter seems to be affected by dosing schedules, duration of therapy and frequency of side effects (Broutet et al., 2000; Veldhuyzen et al., 1998; Hunt et al., 2000; Suebaum et al., 2002).

In the last decade, the approach to the treatment of *H. pylori* infection has changed as the success rate of usual eradication regimens decreased from over 90% to about 80%, a critical threshold below which the eradication rate is considered unsatisfactory (Bergamaschi et al., 2007). In Canada, there is a relatively high resistance rate of *H. pylori* to metronidazole (20-40%) when compared with clarithromycin (<5%) or amoxicillin (<1%) (Fallone et al., 2000). A recent systematic review of clinical trials containing Canadian data and assessing *H. pylori* eradication regimens showed that the success rate of both triple therapies consisting of proton pump inhibitor (PPI), clarithromycin and either amoxicillin or metronidazole for one week were 84% and 82% respectively while the cure rate of PPI, amoxicillin and
metronidazole was 76%. Quadruple therapy of PPI, bismuth, metronidazole and tetracycline, given for 10 days, achieved a success rate of 87% (Rodgers et al., 2007).

In an empirical strategy, the use of PPI, amoxicillin and a new antimicrobial (e.g. rifabutin, levofloxacin or furazolidone) could be used as ‘rescue’ regimen should the conventional triple and quadruple therapies fail. The new antibiotic could be rifabutin or levofloxacin, although, with the use of rifabutin, a reversible myelotoxicity as leukopenia and thrombocytopenia has been described (DiMario et al., 2006).

The decreasing success rate of conventional \textit{H. pylori} eradication regimens has led to exploring novel non antibiotic agents to enhance the efficacy of these regimens. Probiotics have been postulated to be beneficial against \textit{H. pylori}. Probiotics may compete directly with \textit{H. pylori}, possibly through the inhibition of adherence, as well as produce metabolites and antimicrobial molecules. Data supporting the aforementioned effects of probiotics is mainly derived from \textit{in vitro} and animal studies (Franceshi et al., 2007). In addition, probiotics may indirectly improve the success rate of eradication therapy through improving patients’ compliance by reducing the occurrence of antibiotic related adverse events (Lewis et al., 1998).

\textbf{2.1.4 \textit{H. pylori} association with gastric cancer and other gastric lesions}

\textbf{2.1.4.1 \textit{H. pylori} and gastric cancer}

The evidence for a relationship between the infection and gastric cancer is strong enough for \textit{H. pylori} to be classified as class A carcinogen by the World Health Organization (WHO) in 1994. The IARC report was based entirely on epidemiologic evidence since experimental evidence was not available. The evaluation of the carcinogenesis of \textit{H. pylori}
infection was based on human carcinogenesis data from three cohort and nine retrospective case control studies, all of which yielded a higher risk of gastric cancer associated with *H. pylori* infection (IARC; 1994). Before addressing the nature and characteristics of this association, a description of the cancer and its epidemiology is warranted.

**Gastric cancer incidence and mortality**

World wide, gastric cancer poses a heavy burden. Global cancer statistics for the year 2002 reported one million new cases of gastric cancer diagnosed worldwide, accounting for nearly 10% of all new cancers (Parkin et al., 2005). World statistics on stomach cancer mortality and incidence show a distinct geographical variation. Nearly two-thirds of all new cases occur in developing countries. The actual numbers of cases diagnosed will increase each year, because of the aging population. Gastric cancer is ranked second as a cause of cancer mortality in the world, being surpassed only by lung cancer (Prinz et al., 2006).

High risk areas, with age standardized incidence ranging from 30 to 85 cases per 100,000 in men and 15 to 40 cases per 100,000 in women, include Japan, China, Portugal, Peru, Italy, Costa Rica, Ecuador, Columbia, Estonia and Latvia. Low risk areas, with age standardized incidence ranging from 4 to 8 cases per 100,000 in men and 2 to 4 cases per 100,000 in women include Kuwait, Israel and the United States (Parkin et al., 1992).

In Canada, gastric cancer ranks 14th in incidence among the major types of cancer with approximately 2800 new cases reported each year, and ranks 8th with respect to mortality with 1950 deaths per year (Canadian Cancer Statistics; 2008).

**Risk factors for gastric cancer**

Gastric carcinogenesis is a multi-factorial process. Gastric carcinomas result from the interaction of factors related to diet, environment, individual genetic susceptibility and *H.*
*pylori* infection (Parkin et al., 2006). Migrant studies show that gastric cancer rates are initially similar to those in the home country and gradually increase or decrease to fit the profile of the adopted country. This adaptation confirms the importance of lifestyle factors in the etiology of the cancer and also suggests the importance of “environment” in early life in determining the risk for stomach cancer (Prinz et al., 2006).

**Diet and gastric cancer**

Epidemiological studies have been carried out to identify dietary risk and protective factors for gastric cancer. According to the World Cancer Research Fund and the American Institute for Cancer Research, there exists a negative association between consumption of fresh fruits and vegetables and the risk of the disease. On the other hand, cured fish and meat and salt intakes were positively associated with the development of gastric cancer (WCRF; 1997). A meta-analysis of cohort studies showed an inverse association between fruit intake and gastric cancer incidence (RR = 0.82; 95% confidence interval, CI = 0.73-0.93) which became stronger with follow-up periods of > or = 10 yrs (RR = 0.66; 95% CI = 0.52-0.83). For vegetables, the RR was 0.88 (95% CI = 0.69-1.13) using all incidence studies and 0.71 (95% CI = 0.53-0.94) when considering only those with the longer follow-up (Lunet et al., 2005). A suggested mechanism is that the antioxidant content of fruits and vegetables may contribute to the prevention of carcinogenesis in the stomach by neutralizing the reactive oxygen species. A detailed discussion of this mechanism is presented in section 2.3.2.

There is ample epidemiological and experimental evidence for the role of excessive salt intake in the etiology of gastric cancer. A high salt concentration in the stomach destroys the mucosal barrier and leads to inflammation and damage such as diffuse erosion and degeneration, in addition to increasing the mutagenicity of nitrate/nitrite-containing foods
A correlation analysis in Henan, China showed positive correlation coefficients between the salt quantity sold in one area and the gastric cancer mortality in that area. The correlation coefficient for males and females were 0.63 and 0.54, both of which were significant at the 1% significance level (Lu et al., 1987). A recent review by Tsugane et al. (2007) of the evidence from descriptive and analytical epidemiological studies of the association between salt intake and gastric cancer indicated that dietary modification to reduce salt and salted food intake is probably protective against the cancer.

In their review, Terry et al. (2002) evaluated the relationship between alcohol consumption, cigarette smoking and gastric cancer. They found out that smoking moderately increases the risk for gastric cancer (RR: 1.1-2.0). There was no association between alcohol consumption and the cancer.

Among all risk factors, *H. pylori* infection has been consistently shown to increase the risk for gastric cancer. A description of the association between this infection and gastric cancer will be presented next.

**Association between H. pylori infection and gastric cancer**

Numerous retrospective and prospective studies have shown a significant correlation between *H. pylori* infection and gastric cancer risk. A meta-analysis of 16 case control studies with age and sex matched controls showed that *H. pylori* infection significantly increased the risk for gastric cancer by 3 folds (Huang et al., 2003). The results of an earlier meta-analysis by the *Helicobacter* and Cancer Collaborative Group showed that the odds of developing gastric cancer in *H. pylori* infected subjects is 3.0; 95%CI: 2.3-3.8 (*Helicobacter* and Cancer Collaborative Group; 2001). A main strength of this meta-analysis is the fact that it included data from case control studies nested within prospective cohorts to assess the
relationship between \textit{H. pylori} infection and gastric cancer. This is particularly important given the fact that in retrospective studies, \textit{H. pylori} infection is, by necessity, assessed after the development of gastric cancer. \textit{H. pylori} does not colonize areas of cancer, intestinal metaplasia, or atrophy and there is ample evidence that with the development of advanced gastric cancer the organism can be lost from the stomach (Karens et al., 1991; Forman, 1998). A recent meta-analysis of observational studies aimed to assess the relationship between \textit{H. pylori} infection and early gastric cancer showed that \textit{H. pylori} infection is strongly associated with early gastric cancer when compared with non-neoplasm controls (OR: 3.38; 95% CI: 2.15-5.33), or advanced gastric cancer (OR: 2.13, 95% CI: 1.75-2.59) (Wang et al., 2007).

Whether \textit{H. pylori} infection alone causes gastric cancer in humans is not evident. Although this bacterium fulfills the Koch’s postulates for identification as the causative agent for gastritis, the evidence is less convincing for gastric cancer. The postulates are: (i) the organism must be isolated in every case of the disease, (ii) it must be propagated in isolated culture, (iii) products of this culture must be capable of reproducing the disease when inoculated in a non-infected host, and (iv) the same agent must be recovered from this second host. Obtaining evidence for the third and fourth postulates is ethically not accepted in humans; therefore animal models have become very useful. The Mongolian gerbil has been used as an experimental animal model for studying \textit{H. pylori} infection because it can stably colonize and induce severe chronic gastritis, ulceration, and cancer-simulating human diseases in this animal. In contrast, \textit{H. pylori} can only induce mild inflammation in many mouse models (Osawa et al., 2001). Mongolian gerbils showed a severe infiltration of polymorphonuclear and mononuclear cells in the lamina propria and mononuclear cells
infiltration with lymphoid follicle in the submucosa, one month after *H. pylori* inoculation.

Erosion of the gastric mucosa appeared soon after inoculation, whereas gastric ulcers, gastritis and atrophy with globlet cell metaplasia occurred between three and six months after inoculation. This sequence of histologic changes in gastric mucosa is similar to those observed in human beings (Kodama et al., 2005). Many studies have shown a marked increase in the chemical carcinogenesis risk in the presence of *H. pylori* infection, in Mongolian gerbils (Sugiyama et al., 1998; Tokieda et al., 1999; Shimizu et al., 1999). Watanabe et al. (1998) and Honda et al. (1999) inoculated *H. pylori* strains, similar to those found in humans, into 5-week-old SPF Mongolian gerbils. The results showed that 37% and 40% of animals in the first and second study respectively developed well differentiated adenocarcinoma at 72 weeks after inoculation. Sequential histopathological changes leading to gastric cancer were found to be common in both studies and resembled, to a great extent, the histopathological changes observed in human gastric mucosa following *H. pylori* infection. The evidence from the above mentioned experimental studies supported a direct relationship between *H. pylori* infection alone and gastric cancer. This relationship was needed in order to fulfill Koch’s postulates concerning the causative role of the infection in gastric cancer.

Correa (1992) proposed an etiological hypothesis to explain the role of *H. pylori* in progressive tissue and cellular changes in the development of gastric cancer. Figure 2.1 depicts the model proposed by Correa in which he suggested that *H. pylori* infection initially produces superficial gastritis, leading to chronic atrophic gastritis (over decades). Atrophic gastritis may lead to a decrease in acid secretion and consequently to a rise in gastric pH, a condition that favors bacterial growth. Chronic atrophic gastritis may progress to intestinal
metaplasia, a state followed by dysplasia, which may ultimately develop into gastric carcinoma. This model is based on the experience of a cohort of 1422 subjects followed up for an average of 5 years in the high risk of Narino, Columbia (Correa et al., 1990). This complex dynamic flow of precancerous lesions is that of a slow forward movement, but the speed of such movement is not the same in all individuals (Correa et al., 2007).

Figure 2.1. Correa’s model of gastric carcinogenesis (Correa, 1992)

Given the aforementioned model of carcinogenesis, it is logical to assume that eradication of *H. pylori* infection may play an important role in the prevention of gastric cancer. Uemura et al. (2000) reported promising results on the prevention of recurrence of early gastric cancer after *H. pylori* eradication. The results of a systematic review and a meta-analysis of studies investigating the long-term effect of *H. pylori* eradication on gastritis and intestinal metaplasia in the antrum and corpus showed lower OR for gastritis compared to intestinal metaplasia in both sites of the stomach (Rokkas et al., 2007). It seems that
treatment of H. pylori infection might not be beneficial if therapy is given to at risk individuals beyond the “point of no return” when the development of malignancy would progress in an unrelenting fashion despite cure of the infection (De Vries et al., 2007). Future research is needed to determine this “point of no return” in the cascade of gastric carcinogenesis.

As only 1-3% of H. pylori infected patients develop gastric cancer, the risk of gastric cancer for each individual is presumably dependent on the interaction among H. pylori virulence factors, host genetics and environmental factors (Wu et al., 2005). The interplay of polymorphic variants of H. pylori and those of the host is postulated to determine the neoplastic versus non-neoplastic outcome of cell injury.

An important polymorphic virulence factor of H. pylori is the secreted vacuolating cytotoxin, VacA. The protein inserts itself into the bacterial membrane, forming an anion-selective pore (Kim et al., 2004). VacA causes depolarization of the epithelia cell’s membrane potential (Szabo et al., 1999), apoptosis (Peek et al., 1999), inhibition of epithelial cell attachment, and inhibition of T-cell activation. (Gebert et al., 2003). Secretion of VacA protein is associated with the presence of the CagA protein (Covacci et al., 1993). This protein is secreted by some H. pylori strains which were shown to be associated with more severe clinical outcomes, such as peptic ulcer and gastric adenocarcinoma (Parsonnet et al., 1997; Rugge et al., 1999). The cagA gene locus is a marker for the pathogenicity island (PAI), which contain other genes that encode for proteins that form a type IV secretion apparatus, responsible for injecting the CagA protein into the gastric epithelial cells (Backert et al., 2000; Stein et al., 2000). Inside epithelial cells, the CagA protein localizes to the inner surface of the plasma membrane and perturbs the physiological network of intracellular
signaling while causing elevated cell motility with drastic cellular elongation known as the “Hummingbird” phenotype (Amieva et al., 2003). In addition, it complexes with the tight junction proteins such as E-cadherin and disrupts the barrier function of tight junctions in polarized epithelial cells, which leads to the loss of epithelial cell-cell adhesion and promotes degradation of the basement membrane (Hatakeyama, 2007). Infection with CagA positive strains of *H. pylori* is associated with the induction of cytokines such as IL-8, granulocyte-monocyte colony stimulating factor, tumor necrosis factor-α, and nuclear factor-κB (Rieder et al., 1997; Keates et al, 1997; Selbach et al., 2002). *cagE* is another gene located on the cag pathogenicity island and strains of *H. pylori* carrying this gene were linked to an increased production of IL-8 (Covacci et al., 2000). Day et al. (2000) and Fallone et al., (2000) suggested an association between infection with *cagE* *H. pylori* strains and the severity of disease outcomes in both children and adults.

Host genetic factors have emerged as another important determinant of increasing risk for gastric cancer. Cytokines participate in the inflammatory response associated with innate and acquired immunity response. These cytokines are either proinflammatory such as IL-1B, and tumor necrosis factor-α or anti-inflammatory such as IL-10. Genes encoding these cytokines harbor polymorphic regions, which alter gene transcription and thereby influence inflammatory processes in response to *H. pylori* infection (Perez-Perez et al., 2005). The combination of multiple proinflammatory polymorphisms and some anti-inflammatory polymorphisms was found to confer a greater risk for gastric cancer as demonstrated by El Omar et al. (2003).

Given the identified virulence factors of *H. pylori* and the significance of some cytokine polymorphisms of the host in responding to inflammation, it seemed possible to
define a disease risk profile combining host and bacterial genotypes in gastric cancer (Figueiredo et al., 2002). Many studies have confirmed an increased risk for gastric cancer when \textit{H. pylori} CagA strains were found in hosts with genetic polymorphisms that upregulate production of strong proinflammatory cytokines (Figueiredo et al., 2002; El Omar et al., 2003; Zeng et al., 2003).

\subsection*{2.1.4.2 \textit{H. pylori} and peptic ulcer}

Peptic ulcer is a disease of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion. It usually occurs in the stomach and proximal duodenum; less commonly, it occurs in the lower esophagus, the distal duodenum or the jejunum (Ramakrishnan et al., 2007). \textit{H. pylori} infection and the use of non steroidal anti-inflammatory drugs (NSAIDs) are the predominant causes of peptic ulcer disease (Kurata et al., 1997). Patients with \textit{H. pylori} infection have increased resting and meal stimulated gastrin levels and decreased gastric mucus production and duodenal mucosal bicarbonate secretion; all of these factors contribute to the development of ulcer (Ramakrishnan et al., 2007). Although \textit{H. pylori} is present in the gastroduodenal mucosa in most patients with duodenal ulcers, only a minority (10 to 15\%) of patients with \textit{H. pylori} infection develop gastric ulcer disease (NIH consensus conference, 1994). As discussed in the previous section, it seems that virulence factors of the bacterium and host polymorphisms play a major role in determining the outcome of \textit{H. pylori} infection. Eradication of \textit{H. pylori} reduces the relapse rate of peptic ulcer. A recent systematic review and a meta-analysis showed that eradication of the infection increased the proportion of peptic ulcer initially healed and the proportion of patients free from relapse following successful healing. In addition, when symptom relief and occurrence of adverse events were considered, eradication treatment was superior to ulcer-
healing drugs (34 trials, 3910 patients, RR: 0.66; 95%CI: 0.58-0.76) and to no treatment (two trials, 207 patients, RR: 0.37; 95%CI: 0.26-0.53) (Ford et al., 2006).

### 2.1.4.3 *H. pylori* and MALT lymphoma

MALT lymphoma is uncommon and accounts for 5% or less of gastric neoplasms (Al-Mofleh et al., 2004). The median age of occurrence is between 60 and 65 years, with men being affected 2 to 3 times more than women (Al Akwa, et al., 2004).

The normal gastric mucosa is devoid of lymphoid tissue. However, as a reaction to bacterial colonization in the stomach, lymphoid infiltration is triggered (Du et al., 2002). The ongoing exposure of the lymphoid cells to *H. pylori* causes uninterrupted proliferation in an attempt to eliminate the antigen. During this process, the risk of genetic alteration of the cells increases and lymphoid follicles may develop, resulting in mucosa-associated lymphoid tissue (MALT) lymphoma (McCance et al., 2002). The evidence for the association between *H. pylori* and MALT lymphoma is extensive (Wotherspoon et al., 1991; Parsonnet et al., 1994) and has been strengthened by research showing that eradication of *H. pylori* is effective as a first-line treatment for MALT lymphoma. A review of 12 clinical studies with almost 400 patients and case reports have shown that eradication of *H. pylori* infection is associated with complete remission in approximately 80% of patients with low-grade MALT lymphoma in an early clinical stage (Morgner et al., 2000).
2.1.5 *H. pylori* association with esophageal cancer and cardiovascular diseases

*H. pylori* infection, although confined to the stomach, induces a strong host immune response. It is therefore plausible that effects of this response may contribute to the development of diseases in areas other than the stomach. In this section, the possible relationship of this infection with cardiovascular diseases and esophageal cancer will be discussed.

**H. pylori and cardiovascular diseases**

The association between *H. pylori* infection and cardiovascular diseases has been extensively researched in the last few years (Bohr et al., 2007). Among the mechanisms postulated for the onset of *H. pylori*-induced ischemic heart diseases are the systemic increase of inflammatory markers and mediators (Yamaoka et al., 1997), oxidative modifications and changes regarding homocysteine levels (Sung et al., 1996). The evidence from epidemiologic studies regarding the association between *H. pylori* and cardiovascular diseases is contradictory. Pasceri et al. (1998) showed an almost four-fold increased risk of coronary heart disease in cag A seropositive individuals. A significant association between coronary artery disease (CAD) and *H. pylori* was reported by Pieniazek et al. (1999) when 81.5% of coronary heart artery disease patients were seropositive for *H. pylori*, whereas 51% of control subjects were found to be seropositive. A meta-analysis by Pasceri et al., (2006) reported a small but significant association between vascular diseases and CagA-positive *H. pylori* strains. On the other hand, the results of another meta-analysis by Danesh et al. (1999) showed no relationship between *H. pylori* infection and ischemic heart diseases incidence (OR: 1.13, 95% CI: 0.93-1.38). Even the seropositivity for CagA positive strains was
reported to be unrelated to any cardiovascular diseases (Koeing et al., 1999). A recent review by Manolakis et al. (2007) elegantly outlined the contradiction in the literature about the association between *H. pylori* infection and cardiovascular diseases with regard to both epidemiologic evidence and postulated mechanisms of this association. Thus, it appears that the concept of *H. pylori* implication in the pathogenesis of cardiovascular diseases is still open for discussion and further research is needed to settle on the mechanisms of this relationship.

**H. pylori and esophageal cancer**

The possible negative association between *H. pylori* infection and esophageal cancer has been the subject of extensive research. Gastroesophageal Reflux Disease (GERD) is the strongest known factor for developing Barrett’s oesophagus, a metaplasia of the distal oesophagus associated with an increased risk of oesophageal adenocarcinoma (Lagergren et al., 1999). To date there is no consensus in the literature on whether *H. pylori* infection protects from GERD and whether eradication of the bacterium increases the risk of the disease (Hunt et al., 1999). It has been hypothesized that the loss of acid secretion in the stomach due to *H. pylori* infection may reduce the incidence of GERD. Supporters of this hypothesis point to the opposing time trends of gastric cancer and GERD and its complications. The falling incidence of *H. pylori* infection and gastric cancer in developed countries over the past century have been dramatically opposed by a rapidly increasing incidence of GERD and its squalae (Peek et al., 2002). In 1998, El-Serag and Sonnenberg drew attention to the dramatic decline of duodenal ulcer disease in the United States. They attributed this to the fall in the prevalence of *H. pylori* infection. In the same paper they showed that GERD had risen to an even greater extent. They argued that this might also be
attributable to the fall in *H. pylori* infection (El- Serag et al., 1998). Whether there is a true increase in the incidence of esophageal cancer is debatable. Some researchers suggest that the observed trend is a result of an improved diagnostic accuracy that may have resulted in reclassifying some of the cases of gastric adenocarcinoma to esophageal cancer.

A meta-analysis of 20 observational studies and 4134 subjects examining the association between *H. pylori* and GERD symptoms calculated the pooled odds ratio (OR) for the prevalence of *H. pylori* (Raghunath et al., 2003). A mean prevalence of *H. pylori* of 38.2% was observed in GERD subjects, compared with 49.5% in subjects without GERD. The pooled OR was 0.60 (95% confidence interval (CI): 0.47–0.78), indicating that the odds of *H. pylori* infection was nearly two-thirds in individuals with GERD, compared to those without. Although this finding supports the hypothesis of a protective effect of *H. pylori* infection on GERD there was considerable heterogeneity between the studies. Investigation of the potential cause of the difference between studies failed to show any evidence of publication bias or the year of publication. Some evidence was found for a geographical influence, the association being stronger and more consistent in North America and the Far East than in Western Europe (Raghunath et al., 2003).

If *H. pylori* infection reduces the risk for GERD, it would be expected that treatment of this infection will result in increased risk of reflux disease. However reports in the literature fail to show such a relationship. Befrits et al. (2000) showed that duodenal ulcer patients whose *H. pylori* infection was cured, did not show any difference in cumulative risk for developing esophagitis after 2 years of follow up. In 2004, Fallone and his colleagues from Canada demonstrated that there exist no clinically significant differences in clinical or laboratory related GERD manifestations between *H. pylori* infected and non-infected GERD
patients. It seems, unlikely, therefore, that *H. pylori* infection per se protects against GERD (Fallone et al., 2004).

### 2.2 Prevalence of *H. pylori* infection in the world

*H. pylori* may be the most common infectious disease in the world. Nearly 50% of the world’s population is estimated to be infected (Pounder et al., 1995). However, the prevalence and age at infection differ dramatically in developed versus developing countries. In this section, prevalence of *H. pylori* in different parts of the world and in Canada will be presented.

#### 2.2.1 Prevalence of *H. pylori* infection in the developing world

In contrast with industrialized nations, *H. pylori* infection occurs at an earlier age and with a higher frequency in the developing world. While the prevalence of the infection has dropped significantly in many parts of North America and Western Europe, no such decline has been noted in the developing world (French et al., 2003). In many developing countries, the prevalence of *H. pylori* infection exceeds 50% by 5 years of age. By adulthood, infection rates exceeding 90% are not unusual (Go, M. 2002). In a cross sectional study of 569 Bangladeshi children between 2 and 10 years of age, the seroprevalence of *H. pylori* was 42% by 2 years of age and 67% by 10 years of age (Rahman et al., 1998). Similar findings were reported in studies of children from many parts of the developing world including Peru, Gambia and China (Mitchell et al., 1992; Klein et al., 1994; Thomas et al., 1999). In Egypt, *H. pylori* antibodies were detected in the sera of 90% of 200 Alexandrian mothers (Naficy et al., 2000). Fifteen percent of their infants had become infected by 9 months of age. After 3
years of follow up, the seroprevalence of *H. pylori* infection increased to 25% at 18 months and 30% when the children attained 3 years of age (Naficy et al., 2000).

### 2.2.2 Prevalence of *H. pylori* infection in Europe and the United States

In Europe, the prevalence of infection shows strong regional differences in adults and is highest in developing countries. Prevalence of infection was higher in older compared to younger people (Rothenbacher et al., 2003). The following table presents a selection of prevalence studies carried in different regions in Europe.
Table 2.2 Cross sectional prevalence estimates of *H. pylori* infection in adults from various European countries using serology test for the infection.

<table>
<thead>
<tr>
<th>Country, authors</th>
<th>Number, sex and age of participants</th>
<th><em>H. pylori</em> infection prevalence</th>
<th><em>H. pylori</em> infection prevalence in specific age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Ireland Murray et al. (1997)</td>
<td>4742, males and females, 12-64 yrs</td>
<td>50.5%</td>
<td>12-14 yrs: 23.4% 60-64 yrs: 72.7%</td>
</tr>
<tr>
<td>Italy, Dominici et al. (1999)</td>
<td>3289, males and females, 12-66 yrs</td>
<td>59.7%</td>
<td>16-16 yrs: 30.1% 56-66 yrs: 81.9%</td>
</tr>
<tr>
<td>Germany, Seher et al. (2000)</td>
<td>6748, males and females, 18-79 yrs</td>
<td>40%</td>
<td>18-29 yrs: 22% 70-79 yrs: 61%</td>
</tr>
<tr>
<td>England, Danesh et al. (2000)</td>
<td>1025, males, 40-59 yrs</td>
<td>72.2%</td>
<td></td>
</tr>
</tbody>
</table>

In the United States, almost 25% of children 6-19 years old are infected (Staat et al., 1996). In an attempt to assess the seroprevalence of *H. pylori* infection among different ethnic groups in the adult US population, Everhart et al. (2000) analyzed the sera from 7465 adult participants during the first phase of the third National Health and Nutritional Examination Survey (1988-1991). They found the seroprevalence of *H. pylori* among all participants was 32.5% with higher estimates in older individuals: 16.7% for persons 20-29 years old vs. 56.7% for those >70 years old. Age-adjusted prevalence was substantially higher among non-Hispanic blacks (52.7%) and Mexican Americans (61.6%) than non-Hispanic whites (26.2%). Malaty et al. (2002) examined the prevalence of *H. pylori* infection in a cohort of 224 children selected retrospectively from the community of Bogalusa, Los Angeles, USA. The children were followed for 21 years. Eighteen percent of children at age 1-3 years had *H. pylori* antibodies. By 18-23 years, the prevalence of infection was 24.5%.

### 2.2.3 Prevalence of *H. pylori* infection in Canada

Prevalence studies that test a representative sample of the Canadian population are few and generally do not include asymptomatic individuals. In Nova Scotia, in attempt to
estimate the rate of acquisition of *H. pylori*, the seroprevalence of *H. pylori* infection was found to be lower (21%) in the third decade of life than in the eighth decade (50%) in a sample of 316 randomly selected, non-patients subjects aged 18-72 years (Veldhuyzen et al., 1994). No inference about *H. pylori* prevalence in Canada could be made from this study due to the small sample size. There are certain populations in Canada with much higher infection rates. One study found that *H. pylori* was present in the sera of 95% of a First Nations community in Manitoba (Bernstein et al., 1999). The CADET-PE study found approximately 30% of dyspeptic patients were *H. pylori* positive. Infection was assessed by endoscopy, however patients who were willing to participate and refused the endoscopy had a $^{13}$C breath test (Thomson et al., 2003). None of the studies described above aimed to assess the prevalence of *H. pylori* in the general asymptomatic Canadian population. The prevalence estimates that resulted from these studies are specific either to dyspeptic patients (the CADET study) or the Inuit communities in the Canadian Arctic. The results of these studies and the important limitations are summarised in Table 2.3.

In children, *H. pylori* infection prevalence rates vary considerably across Canada. In an unpublished report from the ongoing Prevalence, Symptomatology and Investigation of Pediatric *H. pylori* infection [PSI-PHI] study, prevalence rates of 5.28% (13 of 246) have been observed in children aged 5-18 years. This study involved Canadian academic centers where upper GI endoscopy is performed for upper GI symptoms (Jacobson et al., 2005). Higher seroprevalence rates were observed in indigenous populations: 51% in children from the Arctic communities of Chesterfield Inlet and Repulse Bay, Nunavet (Mc Keown et al., 1999) and 67% in First Nation’s children (aged 6 weeks to 2 years) of the subarctic community, Wasagamack, Manitoba (Sinha et al., 2002).
Table 2.3 Results and limitations of various cross sectional studies of *H. pylori* prevalence in Canada.

<table>
<thead>
<tr>
<th>Author (year) and study design</th>
<th>Location and method of diagnosis for <em>H. pylori</em></th>
<th>Sex and number of participants</th>
<th>Prevalence of various age groups studied</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veldhuyzen et al. (1994); Halifax; serology</td>
<td>Males (129) Females (187)</td>
<td>18-72 yrs: 37.9%; 3\textsuperscript{rd} decade: 21.4%; 4\textsuperscript{th} decade: 27.9%; 5\textsuperscript{th} decade: 39.2%; 6\textsuperscript{th} decade: 41.1%; 7\textsuperscript{th} decade: 47.2%; 8\textsuperscript{th} decade: 50%</td>
<td>Small sample size</td>
<td></td>
</tr>
<tr>
<td>Mc Keown, et al. (1999); Manitoba; serology</td>
<td>Males (111) Females (145)</td>
<td>2-65 yrs: 50.8%; &lt;15 yrs: 32.1% 15-29 yrs: 58.9% 30-44 yrs: 44.2% 45-59 yrs: 50.0% 60-74 yrs: 64.3%</td>
<td>Small sample size; High risk group</td>
<td></td>
</tr>
<tr>
<td>Bernstein, et al. (1999); Wasagamack Manitoba</td>
<td>Males (114) Females (192)</td>
<td>&lt;15 yrs: 100% 15-29 yrs: 94.7% 30-49 yrs: 98.0% 50-69 yrs: 92.5% ≥70: 77.8%</td>
<td>Small size; High risk group</td>
<td></td>
</tr>
<tr>
<td>Thomson, et al. (2003) Across all Canada; histological test of gastric biopsy</td>
<td>Males (552) Females (448)</td>
<td>18-84 yrs: 29.7%</td>
<td>Clinical population: Dyspeptic patients</td>
<td></td>
</tr>
</tbody>
</table>

2.3 Interaction between *H. pylori* infection and vitamins E & C

The facts that approximately half of the world population is infected with *H. pylori*, and only 1-3% develop gastric cancer suggest that the existence of *H. pylori* infection itself does not explain fully the etio-pathogenesis of gastric cancer. Other factors such as diet, genetic background of the host or their interaction may contribute to the development of the disease. In this section, possible interaction between *H. pylori* infection and antioxidants in
the diet will be discussed. The effect of *H. pylori* infection on the oxidative stress of the body on one hand and the antioxidants defense mechanism against oxidation on the other hand are two important arms of the possible interaction between the bacterium and the antioxidants.

### 2.3.1 *H. pylori* and oxidative stress

*H. pylori* itself is not genotoxic; it does not bind with the DNA of gastric cells. Therefore, what triggers the cancer cascade is likely to be a by-product of the infection. A hallmark of *H. pylori* infection is a host inflammatory response which includes infiltration of neutrophils with inducible nitric oxide synthase generation of NO to potentially eradicate the organism (Mannick et al., 1996; Pinto-Santini et al., 2005). Because of this bacterium’s ability to persist, the inflammatory response becomes chronic. The toxicity of NO has been described to act by two chemical processes: (i) oxidation of NO by superoxide leading to the formation of peroxynitrite and nitrosating species and (ii) direct reactions between proteins and NO by nitrosylation events. Beside deamination, NO and/or peroxynitrite can cause DNA base oxidation (Jaiswal et al., 2001). This DNA damage may disrupt the expression and function of several genes (such as p53) and is believed to contribute to the development of gastric cancer (Ernst et al., 1999). Evidence for an increased oxidative stress in the case of *H. pylori* infection comes from both animal and human studies, as will be described below.

In rats, Oh et al. (2005) showed that *H. pylori* infection significantly increased gastric mucosal thiobarbituric acid reactive substances, a marker of lipid peroxidation. Animals treated with vitamin E had significantly lower levels of oxidative stress markers.

In humans, Pignatelli et al. (2001) demonstrated that eradication of *H. pylori* attenuated the oxidative stress in the gastric mucosa; antral biopsies were obtained for 34 patients with chronic atrophic gastritis and peptic ulcer before and after bacterial eradication.
Inducible nitric oxide synthase and nitrotyrosine levels were assessed as markers of production of reactive oxygen species and oxidative damage to proteins, respectively, as well as 8-hydroxy-2-deoxyguanosine as a marker of DNA damage. After successful *H. pylori* treatment the levels of the three markers decreased significantly.

Using $^{15}$N breath test to assess body nitrosation and oxidative stress caused by highly reactive oxygen species and carbenium ion intermediates, Junghans et al. (1999) demonstrated that after ingestion of $^{15}$N$_2$ urea by all participants, patients with *H. pylori* showed significantly increased $^{15}$N enrichments of exhaled N$_2$ compared with healthy volunteers.

### 2.3.2 Vitamins E and C defense system

The human body has several mechanisms to counteract damage by free radicals and other reactive oxygen species. Two important lines of defense are first, a system of enzymes which decrease concentrations of harmful oxidants in the tissues; second, the presence of antioxidants. An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity to damage. Some antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism of the body. Other antioxidants are found in the diet. Although about 4000 antioxidants have been identified, the best known are vitamins C and E (Bagchi et al., 1998). The rest of this section will be dedicated to explaining the role of these two vitamins in defending the body against oxidative damage and to discuss the evidence for such a role in the literature.

Vitamin C or ascorbic acid is a water soluble vitamin. This vitamin interacts with free radicals in the water compartment of cells as well as in the fluids between the cells. It is considered the most important antioxidant in extra cellular fluids (Halliwell, 1996). Vitamin
C is effective in protecting tissues against oxidative damage. Its reactivity with ROS/RNS and concentrations in human fluids and tissues make it a likely scavenger of these species (Halliwell, 1996). Under oxidative stress ascorbic acid is reduced to dehydroascorbic acid (DHAA), and this reaction is reversible. However if DHAA is subjected to further oxidation, it will be transformed in a non reversible reaction to 2, 3 diketogluconic acid, which will be excreted in the bile.

Vitamin C has a sparing effect on vitamin E as it regenerates vitamin E from tocopheroxyl radical after the latter has neutralized free radicals (Halliwell et al., 1999).

Vitamin E is the collective name for eight compounds, four tocopherols and four tocotrienols, found in nature; it is a fat soluble substance found in all cellular membranes and is mainly stored in adipose tissue, the liver and the muscle. Vitamin E is a principal antioxidant in the body. It protects cell membranes from damage by peroxidizing agents such as hydroxyl radicals, alkoxy radicals, peroxyl radicals, singlet oxygen and a number of oxygen-metal complexes. These agents not only damage lipids, but they also produce secondary intermediates that lead to a chain of lipid peroxidation. Alpha tocopherol protects lipids by scavenging peroxyl radicals without reacting in further chain-propagating steps (Cadenas et al., 1984).

In “health” there is a balance between ROS / RNS and the antioxidants defenses; in the presence of infection such as H. pylori or compounds in cigarette smoke, this balance is shifted in favor of the reactive species (Evans et al., 2001).

Given the well-defined role of vitamins E and C in protecting the body against free radicals and the slow progression of precancerous lesions has stimulated cancer chemoprevention trials in many countries. The results of these trials are contradictory and
have led the panel of the American Institute for Cancer Research to classify the evidence for a protective effect of antioxidants intake as no stronger than possible or probable (WCRF, 1997). Below is a discussion of few gastric cancer chemoprevention trials using various combinations of dietary antioxidants.

In Colombia, Correa and his colleagues showed that vitamin C and/or Beta carotene supplementation for 6 years in subjects with confirmed histologic diagnosis of multifocal nonmetaplastic atrophy and/or intestinal metaplasia resulted in a significant regression of precancerous lesions. At 12 years of follow up, the beneficial effect of antioxidants was not detected, suggesting that the effects of antioxidants last only as long as the supplements are used (Correa et al., 2000). In contrast to the previous findings, You et al. (2006) showed that supplementation with vitamin C, E and selenium for 7.3 years had no beneficial effect on the prevalence of precancerous gastric lesions or on gastric cancer incidence. Recently, a randomized double blind chemoprevention trial was conducted in Venezuela (in a high risk population for gastric cancer) to determine the effect of 3 years supplementation with vitamin C, E and Beta carotene on the progression and regression of gastric lesions. The results of this trial confirmed a lack of effect of antioxidants supplementation on the precancerous gastric lesions (Plummer et al., 2007).

In contrast to the conflicting evidence regarding the relationship between dietary supplementation with vitamin E and C and gastric cancer, the association between serum levels of these vitamins and the risk of the disease was more consistent. Choi et al. (1999) examined the relation between serum antioxidative vitamin levels and lipid peroxidation in gastric carcinoma patients, and found that the levels of ascorbic acid in patients with gastric carcinoma were less than one fifth of the controls. Vitamin E levels in the serum of cancer
patients were significantly decreased compared to the control group, but there was no
difference in retinol between the groups. Serum malondialdehyde levels (as a marker of lipid
peroxidation) were significantly higher in the cancer patients than in controls. In Slovakia,
Beno et al. (2000) compared blood levels of natural antioxidants in gastric, colorectal
precancerous lesions and gastric cancer patients with control subjects; they found that the
average levels of vitamin C and E were significantly reduced in all precancerous lesions and
carcinoma groups. Furthermore, in India Arivazhagan et al. (1997) showed that erythrocyte
lipid peroxidation was markedly increased and both enzymatic and non enzymatic-
antioxidants including ascorbic acid and vitamin E were decreased in patients with gastric
cancer when compared to age and sex matched normal subjects. As part of the study of
gastric cancer precursors in Narino, Colombia, blood levels of ascorbic acid, vitamin E,
retinol, prealbumin and carotenoids were measured and correlated with the histopathologic
findings of gastric biopsies. The vitamin E levels in males were significantly lower in
subjects with gastric dysplasia than in subjects with normal mucosa (Haenszel et al., 1985).
Since all of the studies mentioned above had cross sectional designs, it was not possible to
determine whether the observed differences in the vitamins blood levels is a result or a cause
of the disease.

The difference in the relationship between dietary and serum vitamins E and C and
gastric cancer can be explained by the presence of factors that alter the bioavailability of
these vitamins and which the epidemiological studies have failed to control for. The question
whether *H. pylori* infection is one of the factors that alter the bioavailability of vitamins E
and C will be discussed further in the next section.
2.3.3 *H. pylori* infection and systemic levels of vitamins E and C.

In his latest review, Correa (2006) postulated that in the case of *H. pylori* infection, if ROS and RNS overcome the antioxidant defense system, gastric carcinoma may be induced. However if the protective antioxidants succeed in neutralizing the increase in ROS and RNS, cancer may be prevented. Therefore, in certain populations or ethnic groups where oxidative stress is more controlled or less intense, despite presence of *H. pylori* infection, the risk for gastric cancer will be lower. Several studies have examined the effect of *H. pylori* on serum levels of antioxidants (mainly vitamin C), but the results are contradictory.

In 1997, Webb et al. conducted a multi-center study that investigated the relationship between plasma levels of vitamin C and *H. pylori* infection status. There was no significant difference between the serum vitamin C concentrations of the 737 *H. pylori* infected subjects and 666 controls. The authors concluded that there is no association between *H. pylori* seropositivity and plasma levels of vitamin C. Similarly, Phull et al. (1998) failed to establish any correlation between plasma levels of vitamin C, E and A and *H. pylori* seropositivity in dyspeptic patients.

On the other hand, Woodward et al. (2001) showed that the mean plasma vitamin C concentration in those who were *H. pylori* positive was only 65% that of those classified negative. Although partly explained by differences in age, sex, social class, smoking and vitamin C intake, the systemic reduction was observed across almost all subgroups after stratification. Adjustment for all these factors still gave a plasma vitamin C level for *H. pylori* positive individuals which was 80 % that of *H. pylori* negative individuals (p<0.0001). These findings were echoed by Khandzode et al. in 2003, where the authors found a significant increase in serum superoxide dismutase and serum malondialdehyde, and significant decrease
in plasma ascorbic acid in *H. pylori* gastritis and gastric cancer patients as compared to control subjects. In 2007, Tari et al. demonstrated that eradication of *H. pylori* infection resulted in a significant increase in serum vitamin C levels in 16 subjects with early intestinal type gastric cancer.

A possible explanation of the disparities of the results is the lack for control for dietary intakes of the vitamins. Differences in dietary intakes may have masked the effect of *H. pylori* infection on the systemic levels of the vitamins. Another explanation may be the diagnostic method of *H. pylori*, as most of the studies mentioned above used serology to test for the infection. Serological testing does not differentiate between current and past infection, a limitation that may create a bias in the classification of *H. pylori* positive and negative subjects. Therefore, whether *H. pylori* affects the bioavailability of vitamins E and C is still largely unknown.

### 2.4 Summary

The identification and characterization of *H. pylori* as a risk factor for gastric lesions including cancer is considered a milestone in the field of gastroenterology and a major advancement on the way to conquering gastric cancer. In Canada, there is a current debate about eradication of *H. pylori* in the general population as there are still important unanswered questions about the burden of *H. pylori* infection in the country, the safety and practicality of its eradication for the purpose of cancer prevention. The first step resolving this debate, as recommended by Sullivan et al. (2004), is to investigate the prevalence of this infection in the average risk Canadian population. To date, the results of the prevalence
studies in Canada can not be applied to the general population due to limitations in sample size and type of populations studied. The objectives of the first study in this thesis are:

1- To estimate the prevalence of *H. pylori* infection in an adult Ontario population.

2- To investigate the relationship among various lifestyle and dietary factors with the incidence of the infection, including the well documented risk factors for the infection such as age, sex, SES, number of siblings in addition to other still controversial factors such as BMI, intake of fruits and vegetable, incidence of polyps and diabetes and smoking and alcohol intakes.

Another important question in *H. pylori* research that still presents a puzzle for the scientific community is: why only a small proportion of infected subjects develop gastric cancer. This puzzle may be explained by better understanding of the mechanisms of *H. pylori* pathogenecity. Oxidative stress is one of the mechanisms by which *H. pylori* infection may lead to gastric cancer. Recent research demonstrated that *H. pylori* infection increases the production of oxygen and nitrogen reactive species. Dietary antioxidants, mainly vitamins E and C are known to alleviate oxidative stress by neutralizing oxidants. Whether *H. pylori* infection, which leads to this state of oxidative stress, alters the bioavailability of dietary antioxidants including vitamins E and C is still largely unknown. The second study aims to add evidence to the currently limited data on the possible effect that *H. pylori* infection might exert on the relationship between dietary vitamins E and C and their blood levels. The objectives of the second study therefore are

1- To compare the effect of supplementation with vitamin C and E on their serum levels between *H. pylori* infected and non-infected subjects
2- To compare the effect of supplementation with vitamin C and E on marker of oxidative stress in plasma between *H. pylori* infected and non-infected subjects.
Chapter 3: Methods

3.1 Project 1: Prevalence of *H. pylori* infection in Ontario

3.1.1 Study Design

This study is a cross sectional investigation of the prevalence of *H. pylori* infection and its determinants in a sample of Ontario residents. The investigators used blood samples available from controls who participated in the Ontario Familial Colon Cancer Registry (OFCCR), one of six international sites participating in the Co-operative Familial Registry for Colorectal Cancer Studies, established by the US National Cancer Institute. Details of the study sample and data collection are described below.

3.1.2 Ethics approval and funding

This study was granted approval by the University of Toronto Research Ethics Board for one year starting January 20, 2005. Since the samples were stored and later analyzed at Mount Sinai Hospital, approval of the Hospital’s Ethics Board was also required. It was obtained on December 14, 2004 for a period of one year. Copies of the approval letters are in appendices A and B.

In the OFCCR consent form, subjects agreed to the use of their biological specimen and various demographic and risk factor information to help advance research in cancer. Since *H. pylori* is a carcinogen as per the WHO classification, the use of the blood samples to estimate *H. pylori* prevalence was in accordance with the consent form.

The privacy and confidentiality were respected as this project was dealing with anonymous blood samples and epidemiological data. There was no access to the names and
identities of the participants by any member of the research team. Data were stored electronically with password protection to ensure that only authorized personnel had access to it. Any report was presented in aggregate form and no individual participant was identifiable from the data presented.

Funding was provided by Astrazeneca, a large pharmaceutical company with research involving many therapeutic areas, including gastrointestinal.

3.1.3 Methods of the OFCCR

From year 1997 and up to date, the OFCCR attempted to collect detailed family history information, epidemiologic data, blood samples and tumor tissues from all incident colorectal cancer patients, their families and controls. The main purpose of the OFCCR is to facilitate future lower digestive system investigations in such areas as genetic epidemiology, gene discovery, primary prevention, psychological research, screening and treatment.

Ascertainment of the OFCCR controls

Population controls were identified using a random selection from Infodirect, a service of Bell Canada that provides a list of residential telephone numbers in Ontario and the Year 2000 Ministry of Finance Assessment file. Potential respondents were contacted by phone in order to describe the study and identify eligible and interested people. A trained phone interviewer called potential participants from Monday to Saturday during various times of the day. When contact was made, questions were asked to identify all household members aged 20 to 74 years as of 1st of July 1999, residing in Ontario, never diagnosed with colorectal cancer and able themselves or through a proxy to communicate in English. Recruitment of controls took place in two phases carried out in 1999 and 2002-2003. If there was more than one eligible household member, then one person was randomly selected and
asked to participate. If an eligible participant was not available, a callback was made at a different date and time. Calls were recycled among the call periods for a total of five attempts. After six weeks, three further attempts at contact were made. In total, eight attempts were made per household. After the eighth attempt, if no contact was initiated, the household was classified as a non responder.

In year 1999, 891 controls were recruited to match the cases by sex and 10 year age groups. They were selected from the general population of Ontario, using a random selection from Infodirect. Blood samples were requested 3 years post recruitment. From year 2002 to 2003, 1046 controls were matched to cases by sex and 5 year age groups from the general population of Ontario, using Year 2000 Ministry of Finance Assessment File. Blood samples were requested at the time of recruitment.

In year 2007, of the 1,937 subjects recruited during both phases, a total of 1313 (68.4%) gave a blood sample for research.

**Blood sample collection for OFCCR controls**

Eligible population controls were mailed a package including:

- Cover letter explaining the purpose of the study and intention of the package
- Family history questionnaire, which collected information on family members, vital status and disease history including mother, father, brothers, sisters and children
- Personal history questionnaire which collected data on bowel screening, medical conditions, medication use, diet, reproductive factors, physical activity, sociodemographic factors and anthropometric measures.
• Diet questionnaire which collected data on usual eating habits 2 years prior to the data collection. Information was obtained about the frequency of intake as well as the serving size.

• Blood sample request/consent

• A postage paid return envelope

Once the control consented to give blood, a blood kit was sent containing the following:

• Cover letter explaining the purpose of the kit

• Instruction sheet

• Four Acid Citrate Dextrose (ACD) glass tubes

• Leak proof test tube envelope

• Prepaid return courier waybill

Each participant was asked to arrange for blood to be drawn at a local laboratory or physician’s office and to mail back the blood kit.

**Blood samples preparation**

Between March 2001 and April 2004, blood samples were shipped, in ACD (Acid Citrate Dextrose) glass tubes, at room temperature by Purolator services to the Biospecimen Repository at the Mount Sinai Hospital. Upon arrival, the samples were centrifuged by an Allegra 6 Bechman (2000 rpm for 10 minutes at 20°C) and then stored in liquid Nitrogen at -180°C. The time interval between blood collection and storage did not exceed 72 hours.

**3.1.4 Methods of testing OFCCR controls for *H. pylori* infection**

For the purpose of this study, which took place in 2004, 1307 blood samples were available. Six blood samples reached the repository after this project ended. The prevalence of *H. pylori* infection was established in blood samples from the OFCCR controls. Of the
1307 subjects who gave blood for the OFCCR, one subject failed to complete the Personal History Questionnaire and the Diet History, therefore his blood sample was discarded. The mean age of the blood givers among controls in the OFCCR was 64.1 ± 8.7 years. The table below presents the distribution by age and sex of blood givers from the control sample.

Table 3.1.1 Distribution by age and sex of blood givers from the control sample of the OFCCR.

<table>
<thead>
<tr>
<th>Age</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60 years</td>
<td>218</td>
<td>191</td>
<td>409</td>
</tr>
<tr>
<td>60-70 years</td>
<td>321</td>
<td>243</td>
<td>564</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>194</td>
<td>139</td>
<td>333</td>
</tr>
<tr>
<td>Total</td>
<td>733</td>
<td>573</td>
<td>1306</td>
</tr>
</tbody>
</table>

The samples were thawed and aliquotted in March 2005. They were put in -80 degrees freezer overnight before being transferred to room temperature for thawing. Ten microliters volume of plasma from each sample was transferred into duplicate 96 well plates. These plates were sent to the Pathology and Laboratory Medicine Department at Mount Sinai where they were stored at 4 degrees to be analyzed for *H. pylori* infection the next day. *H. pylori*-specific IgG antibody titers were measured by an Enzyme-Linked Immunosorbent Assay (ELISA) using the DRG ® screening kit in the robotics lab at Mt Sinai Hospital. Quality control samples with confirmed *H. pylori* status provided with the kit were used in each plate to ensure the precision of the test and to obtain performance data for this kit which showed a sensitivity and specificity compared to reference ELISA of 99.0% and 97.0% respectively (Azar Azad, personal communication, February, 2005). It was not possible to validate the kit in the study sample against gold standards such as histology or culture of
gastric biopsies because limited serum volume (10 ul) was the only biological specimen of
the OFCCR controls sample available for use in this study.

### 3.1.5 Definition of variables

For the purpose of this study, selected variables from the Personal History
Questionnaire were used (copy of this questionnaire is found in Appendix C). The selection
of these variables was based on the scientific literature of factors associated with *H. pylori*
infection. All of these variables were categorical. Below is a description of these variables,
their categories and the number of missing cases in each variable.

Table 3.1.2 Description of the variables included in the analysis, their categories and the
number of missing cases in each variable.

<table>
<thead>
<tr>
<th>Name of the variable</th>
<th>Description</th>
<th>Number of missing responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male or female</td>
<td>None</td>
</tr>
<tr>
<td>Marital status</td>
<td>1-“married”: currently married and living as married 2-“unmarried”: separated, divorced, widowed, singles and never married.</td>
<td>15</td>
</tr>
<tr>
<td>Place of birth</td>
<td>1-In Canada; 2-Outside Canada</td>
<td>0</td>
</tr>
<tr>
<td>Age at immigration</td>
<td>Refers to the age at which the subject immigrated to Canada, 1-&lt;20 years; 2-&gt;20 years</td>
<td>12</td>
</tr>
<tr>
<td>Number of siblings</td>
<td>1-&lt;2 siblings; 2-2-4 siblings; 3-&gt;4 sibling</td>
<td>146</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1-White: white Caucasian; 2-Non-white: blacks (from Africa, Caribbean and North America) and those from the Middle East and Asia</td>
<td>0</td>
</tr>
<tr>
<td>Name of the variable</td>
<td>Description</td>
<td>Number of missing responses</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Income</td>
<td>1-&lt;20 K; 2- 20-40K; 3- &gt;40 K</td>
<td>484</td>
</tr>
<tr>
<td>Education</td>
<td>1- “Low”: high school or less; 2- “Moderate”, technical or college ; 3- “High” Bachelor’s degree or greater</td>
<td>18</td>
</tr>
</tbody>
</table>
| Use of antacids/multivitamins (MVT)/ Acetaminophen (ACE) | 1-“Regular”: using antacids at least twice a week for more than one month; 2-“Not Regular” | Antacids: 14  
Multivitamin: 15  
Acetaminophen : 15 |
| Fruit/ vegetables/meat intake | Referred to diet 2 year prior to completion of the questionnaire.  
1-<1 serving/day; 2- 1-2 servings/day; 3- >2 servings day | Fruits: 48  
Vegetables: 26  
Meat: 24 |
| Alcohol consumption  | The alcohol consumption for subjects between 30 and 40 years, 41 and 59 years and 60 or older referred to the total number of servings of alcohol consumption during the respondents’ 20’s, 30’s and 40’s, and since they turned 50 respectively:  
1-Never; 2- <10 drinks/week; 3- >10 drinks/week | 65                           |
### Table 3.1.2 cont’d

<table>
<thead>
<tr>
<th>Name of the variable</th>
<th>Description</th>
<th>Number of missing responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of smoking</td>
<td>Any year during which the subject smoked at least one cigarette a day for 3 months or longer was counted as one year of smoking 1-Never smoked; 2-&lt;10 years; 3- 10-25 years; 4- 25-40 years; 5-&gt;40 years</td>
<td>71</td>
</tr>
<tr>
<td>Number of cigarettes smoked</td>
<td>Had 4 categories 1- Never smoked; 2- &lt;10 cigarettes/day; 3- 10-20 cigarettes/day; 4- &gt;20 cigarettes/day</td>
<td>53</td>
</tr>
<tr>
<td>Incidence of polyps /Inflammatory Bowel Disease (IBD)/Cancer</td>
<td>1-“yes”: the doctor ever told the patients he had the disease; 2- “No”</td>
<td>Polyps: 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IBD: 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cancer: 16</td>
</tr>
</tbody>
</table>

### 3.1.6 Power calculation

The calculations for the sample size based on 20%, 30% and 40% estimates of prevalence for various confidence intervals are presented in the following table.
Table 3.1.3. Sample size required for selected widths of 95% confidence intervals with 20, 30 and 40% prevalence of *H. pylori* infection (Lenth, R. V. (2006)).

<table>
<thead>
<tr>
<th>Prevalence estimates</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>±2%</td>
<td>±3%</td>
<td>±4%</td>
<td>±2%</td>
</tr>
<tr>
<td>±3%</td>
<td>±3%</td>
<td>±4%</td>
<td>±2%</td>
</tr>
<tr>
<td>±4%</td>
<td>±3%</td>
<td>±4%</td>
<td>±2%</td>
</tr>
<tr>
<td>Number of subjects needed</td>
<td>1537</td>
<td>683</td>
<td>384</td>
</tr>
<tr>
<td>2016</td>
<td>896</td>
<td>504</td>
<td></td>
</tr>
<tr>
<td>2304</td>
<td>1024</td>
<td>576</td>
<td></td>
</tr>
</tbody>
</table>

Thus the availability of 1306 blood samples allowed a width of the 95% confidence interval not greater than ±3% at a prevalence of 30%.

3.1.7 Data analysis

The prevalence of *H. pylori* infection was estimated for each sex separately. Weighted prevalence estimates were obtained using weights calculated as described below.

Weight= Actual percent / sample percent

Where:

Actual percent= Number of individuals in the specified age group in the real population

Total number of individuals in the real population

Sample percent= Number of individuals in the specified age group in the sample population

Total number of individuals in the sample population

In weighting, the distribution of the Ontario population (2003) by sex and 5 year age group was used as reference (Statistics Canada, 2003). The relationship between the prevalence of infection and socio-demographic factors was assessed by estimating age-adjusted odds ratios (OR) and 95% confidence intervals (CI) using logistic regression with infection status as the outcome measure. In all the regression analyses, age was used as a categorical variable because the reference population used for weighting our estimates was
grouped by age and sex. Inter-variable correlations were evaluated prior to multi-variate modeling. Place of birth and age at immigration were significantly correlated (r=0.98); thus a new variable was defined: born in Canada, immigrated to Canada younger than 20 years of age, and immigrated to Canada 20 years of age or older.

In multivariate analyses, the dependent variable was seropositivity for *H. pylori* and covariate variables included available socio-demographic and lifestyle factors. No weighting was used. All modeling was age- and sex-adjusted. Variables were put in the model in order of strength of their association with *H. pylori* infection in the age-adjusted analysis and their importance in the literature. The effect of each variable on the model was assessed and this variable was kept in if it significantly contributed to a better fit of the model. In case of collinearity, the variable which, according to the literature, is unlikely to be biologically significant was taken out of the model. Stratified analyses suggested potential effect modification by sex of the association between *H. pylori* seropositivity and various factors, thus interaction terms for such factors were tested in multivariate logistic models. A test for trend was conducted for variables with more than 2 categories either by logistic regression analysis with the original continuous variable and age as independent variables and infection status as dependent (as in the case of number of siblings) or by a chi-square test when the original variable was categorical (as in the case of education). For all the variables, missing data were put in a separate category and not reported. One case was dropped due to missing age. Most of the data analysis was performed using SPSS version 12.1 (SPSS, 2003). We used STATA version 8.0 (STATA, 2003) in order to calculate the weighted prevalence estimates and their 95% confidence intervals.
3.1.8 Methodological choices

**Cross sectional design and use of the OFCCR stored blood samples**

Two main limitations are frequently cited with cross sectional designs. First, since most characteristics of interest, including infection rates, vary with time as well as across populations, any mention of prevalence ought to be accompanied by a specification of whom and when. Therefore, in this study the prevalence of *H. pylori* is specified for adults and in Ontario. Second, cross sectional designs do not provide direct, time-ordered evidence, as would be available from a prospective study. Instead, in seeking out causal connections, researchers have to rely on respondents’ recollection of key conditions or other indirect information. For the purpose of this study, epidemiologic questionnaires completed by the subjects provided information on basic demographics, medical history and dietary intake.

As stated earlier, out of 1931 subjects who completed the epidemiologic questionnaire, 1313 gave blood samples. A total of 1306 out of the 1313 were tested for *H. pylori* infection. Seven specimens reached the Mt Sinai Hospital Repository after the period during which this study took place.

The use of the OFCCR stored blood samples was a strategic decision for two main reasons. First, there existed no prevalence estimates for *H. pylori* in Canada for the age groups represented in the OFCCR control population. As discussed in Chapter 2, few prevalence studies of the infection have been carried out in Canada, none of these studies looked at adults older than 50 years. Given that most cases of gastric cancer are diagnosed after the mid 50’s and that the average age of those with the disease is 72 years old, it was optimal to have an estimate of *H. pylori* prevalence in adults older than 50 years. The mean age of the OFCCR controls was 64.1 years ± 8.67. Second, the availability of the OFCCR
blood samples presented a rare opportunity to use efficiently available resources while serving the objective of the OFCCR of advancing research in gastro-intestinal cancers.

**Serology for H. pylori infection diagnosis**

The reference method for the diagnosis of active *H. pylori* infection is esophago-gastro-duodenoscopy with gastric biopsies. Given the availability of the blood samples, serological testing was the method of choice to detect *H. pylori* infection in this study. It is less expensive than most other diagnostic methods (discussed in section 2.1.4). With the development of new commercial kits, the sensitivity and specificity of serology has improved and fall in the 90 to 97% range. Serological testing to diagnose *H. pylori* infection, being a non-invasive, inexpensive method with a high sensitivity and specificity, has been widely used in population based studies (Bleeker et al., 1995).

**Robotics services instead of manual laboratory in H. pylori serological diagnosis**

The robotics laboratory at the Mount Sinai Hospital offered many advantages over the Pathology and Medicine manual laboratory. The availability of only 10 micro liter of plasma from the OFCCR blood samples made the robotics lab the most appropriate method. Manual testing requires a higher volume of sample to carry out the ELISA to detect *H. pylori* antibodies. Automated procedures are known to have a decreased personnel and operating costs, less human intervention and fewer laboratory errors, more rapid processing of samples and recording of results, increased safety, better control of the entire process, and decreased need for laboratory space (Amphlett et al., 1991).
3.2 Part II: Effect of *H. pylori* infection on the bioavailability of vitamins E and C.

3.2.1 Study design

This study aimed to assess the effect of *H. pylori* infection on the bioavailability of vitamins E and C, defined as the increase in plasma levels of these vitamins following their oral intake. Volunteers from the University of Toronto students and staff were stratified according to their *H. pylori* infection status. They received vitamins E and C supplementation for a period of 4 weeks. Baseline and post supplementation measurements included plasma levels of vitamin E and C. In addition, plasma oxidative stress was assessed by measurement of plasma malondialdehyde (MDA) and plasma thiols. The differences between post and pre supplementation blood levels of the vitamins, MDA and thiols were compared between *H. pylori* negative and positive groups. Below is a schematic representation of the study design:

Figure 3.2.1. Schematic representation of the study.
It is important to acknowledge that the design of this study has elements of both an intervention trial and a cohort study. Because it involves giving supplements to the subjects, it may be called an intervention. However, the stimulus or the variable of interest that is different among the 2 groups (H. pylori infection status) is not the intervention (supplementation) and can not be manipulated by the researcher; rather it is a characteristic of the subjects in the study, and therefore following these subjects over time is a hallmark of a cohort design.

Originally, a placebo arm was planned for each group. Later during the development of the protocol, a strategic decision was taken to drop the placebo arms for the following reasons: first, the main objective of this trial is to compare pre and post supplementation levels of vitamins between the 2 groups and is not to assess the effect of supplementation on blood levels of the vitamins. Second, there is no evidence that the effect of placebo is
different among the *H. pylori* positive and negative subjects. Given the aforementioned reasons and the burden on the feasibility of the study of increased cost and longer time associated with the recruitment of another group of *H. pylori* positives, it was decided that dropping the placebo arms had no effect on the objective and results of the trial.

In this study subjects did not know their infection status until the end of their participation, however infection status was known to the study coordinator for the purpose of stratification. In order to minimize the study bias, laboratory personnel responsible for blood analyses and the food record analysis technician were kept blinded to the infection status.

### 3.2.2 Ethics approval and funding

This study was approved by the University of Toronto Research Ethics Board in July 2006. Several amendments to the original protocol regarding the recruitment methods and the place of birth and age of immigration of volunteers were later approved; copies of the approval letters are found in appendices D and E. Since the visits and blood tests were carried at the Toronto General Hospital, approval of the University Health Network Research Ethics Board was also obtained. No clearance letter from Health Canada was required because the vitamins were used in accordance with the instruction on the bottles.

All information obtained during the study was held in strict confidence. Each volunteer was assigned a unique study identifier (ID) between 1 and 200. Only this ID was used in the study. IDs will be destroyed 7 years after the date of completion of the study. All records for the volunteers were stored in locked filing cabinets. No names or identifying information will be used in any publication or presentations. No information identifying the subject was transferred outside the investigators in this study. No files with personal identifiers were
transferred electronically. At the end of the study, all \( H. pylori \) positive participants were referred to their family physician for treatment.

Funding for this trial was first sought from the Canadian Cancer Etiology Research Network which provided $33,516. Later, more funds were needed to cover for the urea breath test (UBT) for \( H. pylori \), which is more expensive than serological testing. Serology was originally intended to be the method to diagnose \( H. pylori \); however Toronto General Hospital no longer performed this test starting year 2006. AstraZeneca and Jamiesons Laboratories each provided $5000 to cover the difference in cost between serology and UBT for \( H. pylori \).

3.2.3 Study subjects

Recruitment

Flyers asking for research volunteers were posted in various departments of the University of Toronto in addition to common places such as libraries, student lounges, cafeterias and prayers spaces. Advertisements were also posted on myutoronto.ca and careers.utoronto.ca websites (a copy of this flyer is in Appendix F). Recruitment of volunteers took place between September, 2006 and June, 2007. Since \( H. pylori \) negative subjects were more prevalent in the population studied, and in order to avoid seasonal variation of food consumption, \( H. pylori \) positive and negative subjects were enrolled simultaneously over the period of recruitment.

Inclusion and exclusion criteria

Below is a description of the inclusion and exclusion criteria that were followed during recruitment of volunteers:
Inclusion criteria

1- Age between 18 and 45 years. 

*H. pylori* infection has a long latent period before clinical symptoms develop (between 20 to 30 years). Therefore it is important to understand the pathological mechanisms of this pathogen in adults aged 18 to 45 years.

2- Born Outside Canada (except North America, Western Europe and China) and immigrated to Canada at 10 years or older.

At the start of the trial, recruitment was not restricted to specific places of birth. Two months later, however, only two individuals were *H. pylori* positive among screened volunteers. According to Naja et al. (2007), immigrants to Canada have a higher prevalence of *H. pylori* infection. *H pylori* infection is more frequent in less developed Asian countries like India, Bangladesh, Pakistan, and Thailand than in more developed Asian countries like China (Singh et al., 2006), therefore, to increase the rate of recruitment of *H. pylori* positive participants, Chinese immigrants were excluded.

Exclusion criteria

Exclusion criteria were chosen to limit the variability in factors, other than intake of vitamin E and C, which would affect bioavailability of these vitamins by influencing the oxidative stress pathway. These factors included smoking, high body mass index, strenuous exercise and alcohol consumption of more than 3 drinks/day (Lee et al., 1998; Vina et al., 2000; Darvall et al., 2007). Since blinding for infection status was necessary, subjects who had been treated for *H. pylori* or had partial/total gastrectomy or a history of gastritis were excluded.
Subjects were excluded who were:

1- Current smokers
2- Had a body mass index below 18 or above 25 kg/m²
3- Taking antioxidants supplementation, proton pump inhibitors (PPI) or antibiotics in the previous 4 weeks
4- Training in an athletic team
5- Drinking more than 3 servings of alcohol/day
6- Previously treated for H. pylori infection
7- Had a history of partial or total gastrectomy
8- Had a history of gastritis

Compensation

There was no cost for the volunteers to enroll in the study and the participant was not charged for any research procedure. Each volunteer received $50 for participating in the study to compensate for the time and traveling expenses once they completed the study. If the volunteer came for the first visit, but the Urea breath Test results showed that he/she belonged to a group (H. pylori positive or negative) that already had enough participants, then the subject was compensated with $10 and dismissed from the rest of the study. If the volunteer attended the first and second visits of the study but was not able to come to the third visit for unanticipated reasons, he/she was compensated with $25.

3.2.4 Urea Breath Test for the diagnosis of H. pylori infection

H. pylori infection status was determined by ¹³C urea breath test (UBT) using the commercially available kit: Helikit™. Mock et al. (1999) conducted a validation study of the
Helikit™ in a sample of Canadian (98) and Korean (107) subjects. The results of the Helikit™ were compared to histological examination of gastric biopsies obtained from two separate sites of the stomach. The sensitivity and specificity of the Helikit™ were 93.5%, 95%CI: 88.5-98.5% and 97.3%, 95% CI: 94.3-100%, its overall diagnostic efficiency was 95.6%, 95%CI: 92.8-98.4%. The principle of this test relies upon the capacity of *H. pylori*, when present in the stomach, to hydrolyze orally administered labeled urea to produce isotopically labeled CO$_2$ which diffuses into the blood, is excreted by the lungs and can be detected in the breath samples by means of measuring equipment. Urea can be labeled with 2 different carbon isotopes: $^{14}$C and $^{13}$C. The main difference between these isotopes is that $^{14}$C is radioactive and the $^{13}$C is stable. $^{13}$C is a non radioactive isotope that can be used safely for detecting *H. pylori* in children and women of childbearing age. The Helikit™ uses $^{13}$C as the tracer. In order to avoid false negative results, subjects must not have taken Proton Pump Inhibitors (PPIs) or antibiotics for at least 4 weeks prior to the UBT. In addition participants were instructed to fast for at least four hours prior to the test. Three steps are involved in the collection of breath samples: first, a basal breath sample is collected into a green capped collection tube; second, subjects drink a $^{13}$C urea solution and wait for a period of 30 minutes during which the subject does not eat or drink; third, a second breath sample is collected in a yellow capped collection tube. The breath samples are stable up to six months from the collection date, if stored between 15°C to 30°C (Colaiocco et al., 1998). The breath samples were sent to Medical Diagnostic Services laboratories in British Columbia for analysis. Results were sent by mail and posted on MDS results website: [www.ilanlink.com](http://www.ilanlink.com)
3.2.5 General protocol

In this section, the flow of the trial will be described in detail. Volunteers, interested in participating in the study, contacted the project coordinator either by telephone or email. During this first contact the project coordinator ensured that the volunteer satisfied the aforementioned inclusion/exclusion criteria listed. An appointment was set for the first visit and the volunteer was asked to come after a minimum of 4 hours fast. All visits took place at the Toronto General Hospital General Surgery clinics.

Pre-study visit (visit 1)

The project coordinator met the subjects and described the study objectives and procedures and answered their questions. Subjects agreeing to participate in the study were asked to sign the consent form. A copy of this consent form was given to them. Subjects completed a brief questionnaire that was designed to capture basic demographic information. Copies of the consent form and the questionnaire are found in appendices G and H. The project coordinator administered the UBT and collected the breath samples, which were delivered to MDS Diagnostic Services Laboratories for analysis within 48 hours of collection. A description of the Helikit™ and the steps involved in the UBT, as outlined by the manufacturer, is found in appendix J. Breath samples are stable for six months at room temperature (Colaiocco et al., 1998).

During this visit, the project coordinator instructed the subjects on how to complete a 7-day food record. Subjects who continued the study were asked to bring the 7-day food record to their next visit, covering the 7 days prior to visit 2. When the results of the breath test were available and if there was still a vacancy in the group to which the subjects
belonged (H. pylori positive or negative), they were invited to continue the study. If not, they were mailed $10 compensation and thanked for their interest in the study. For all subsequent visits, the project coordinator called the volunteers in advance to remind them of their appointment and to come in a fasting state.

**Baseline measures (visit 2):**

During visit 2, subjects had blood drawn from the arm (40 ml) at the Toronto Medical Laboratories in Toronto General Hospital. All blood draws were conducted after an overnight 12-hour fast. Venous blood was drawn into EDTA vacutainers and centrifuged at 19°C for 10 minutes at 2400 rpm. The plasma was removed immediately and aliquoted into cryovials which were stored at -70°C. The time between blood draws and freezer storage of the aliquots did not exceed 1 hour.

Subjects were asked to take vitamin E and C supplements daily (in the form of tablets) with a meal in the morning. They were provided with 100 pills of each vitamin and were asked to bring their leftover pills to the next visit. The study coordinator collected the food records belonging to the week preceding this visit (week 0) and instructed the volunteers to complete the second set of 7-day food record during the week prior to the next visit.

**Follow up measures (visit 3):**

Subjects brought their 7-day food records and the left-over pills to visit 3. They had blood drawn from the arm (40 ml). Blood samples were processed as per the second visit blood processing procedures described above. Subjects were given $50 compensation and told to visit their family physician if they had H. pylori infection.
3.2.6 Dietary intake assessment

Seven-day food records were used to estimate total energy, macronutrients and micronutrients, including vitamin E and C intakes, before the first and second blood tests (week 0 and week 4 of the study). Below is a description of this method.

Food intake was recorded by the subject at the time the foods were eaten. During the first visit, subjects were trained in methods of keeping complete and accurate food records. This face-to-face training included a discussion of the significance of the research and the importance of the dietary information. An eight-page form was given to the subjects on which to record all the food and beverages consumed for the period of 7 days (appendix J). The first page had the instructions and an example illustrating the reporting method. Each of the remaining 7 pages corresponded to one record day. The form is designed to capture the nature of the food, its description, the method of preparation, the brand name and the amount consumed. In order to help subjects describe the portion size accurately and in order to decrease inter-subject variability in serving size estimation, a two-dimensional food portion visual chart was used. A copy of this chart is found in appendix K. This chart has been validated for use with adult men and women 20 to 70+ years of age as part of the Framingham Heart Study (Millen et al., 1996). The dietary data were analyzed using Diet Analysis Plus version 5.1 (ESHA Research, 2001). Immediately after collection, the food records were carefully reviewed to ensure an adequate level of detail in describing foods and food preparation methods. If this could not be done in a face-to-face interview, subjects were contacted by telephone to obtain additional detail or to clarify ambiguous information.
3.2.7 Laboratory analyses

Blood analysis took place in the summer of 2007 (July, 07 to September, 07). Blood samples were transferred from Toronto General Hospital (where they were stored) to the Medical Sciences Building at the University of Toronto (where analysis took place). Blood analyses included determination of plasma concentrations of ascorbic acid, alpha tocopherol in addition to indices of oxidative stress. For the purpose of this study, plasma MDA and plasma thiol groups were used to assess the oxidation of lipids and proteins respectively.

Blood analyses were carried out by Ellie Aghdassie, PhD, RD, Research Associate at Dr. Johane Allard’s research laboratory at the University of Toronto. A brief description of the assays used for these analyses are presented in the following section. More details about these assays are found in appendix (L)

Plasma Ascorbic acid.

For the assessment of plasma ascorbic acid, many instrument-based analyses including fluorometry, HPLC, polarography and enzymetic methods were reported in the literature. But due to their inherent limitations, these techniques are not commonly used for routine analyses. However, photometric methods are particularly attractive because of their speed and simplicity. Consequently a large number of such procedures have been developed (Arya et al., 1998). Moeslinger et al., 1995 showed that spectrophotometric determination of ascorbic acid and dehydro ascorbic acid had an analytical recovery of ascorbic acid added to plasma in the range of 93-105% and a between-day variance < 7%.

For this study, plasma ascorbic acid was determined by spectrophotometry as described by Bessey et al., 1947. In this method, total biologically active ascorbic acid,
including dehydro-ascorbic acid (DHAA) concentration was determined spectrophotometrically at 521 nm with 2,4-dinitrophenylhydrazine as the chromogen.

**Plasma Alpha Tocopherol**

Quantitative determination of alpha tocopherol in plasma is commonly performed by high performance liquid chromatography (HPLC) using a reverse phase column (Teissier et al., 1996). The within-day and between day reproducibility of this method were 96.0 and 94.2% respectively. In addition its analytical recovery was 86% (Karpinska et al., 2006). For the purpose of this assay, Varian® HPLC equipment was used including the diode array detector Prostar model 330, the solvent delivery Prostar model 230, the auto-sampler Prostar 410 standard and column OmniSpher C 18. The protocol used in the assessment of plasma alpha tocopherol in this study is described by Natta et al., 1988.

Lipid and alpha tocopherol concentrations in plasma are positively correlated. Therefore an adjustment of alpha tocopherol concentration to lipid content in plasma is warranted in order to decrease any confounding effect that the lipids may exert on the vitamin concentrations (Willet et al., 1998). The lipid fraction of the plasma was extracted by a mixture of chloroform and methanol according to the protocol described by Folch et al., 1957. This method allowed the extraction of more than 95% of plasma lipids (Folch et al., 1957). Plasma concentration of alpha tocopherol adjusted for lipids was expressed as mg/g lipid.

**Plasma malondialdehyde (MDA)**

A frequently used biomarker that provides an indication of the overall lipid peroxidation levels is the plasma concentration of malondialdehyde (MDA), one of several byproducts of lipid peroxidation processes (Church et al., 1985). In this study, plasma MDA
is quantified by the thiobarbituric acid (TBA) test. TBA, when heated under acidic conditions, reacts with a number of chemical species called thiobarbituric acid reactive substances (TBARS) to produce a pink chromophore. Malondialdehyde (MDA) is the major TBARS. The MDA/TBA complex, resulting from the reaction of MDA with TBA, can be quantitated based on intensity of fluorescence at 532 nm or by HPLC. HPLC-based TBA tests improve the specificity of the TBARS test by separating the MDA/TBA complex from other compounds with absorbance at the same wave length (Hwang et al., 2007). The HPLC used in this study for MDA assessment was according to the protocol described by Seljeskog et al., 2006. For this method, the coefficient of variation within run and between runs was 4.1% and 6.7%, and analytical recovery was 90-94% (Seljeskog et al., 2006)

**Plasma thiols**

Essentially all of plasma sulfhydryl (SH) groups are protein associated. These groups are susceptible to oxidative damage and are often low in patients suffering from diseases such as coronary artery disease and rheumatoid arthritis (Wayner et al., 1987). In this trial, oxidative stress was assessed by the spectrophotometric quantification of reduced thiol (-SH) groups using the 5, 5’ dithio-bis (2 nitrobenzoic acid) as described by Hu M., 1988. The reproducibility of this assay is 97.8% (Jenkins et al., 2006).

### 3.2.8 Sample size and power calculations

In order to detect a difference between *H. pylori* negative and *H. pylori* positive groups of at least half a standard deviation in the outcome measurements (the differences between post and pre supplementation serum concentrations of vitamins C and E and measures of oxidative stress) at a power of 80% and a type I error of 5%, 32 subjects were
needed in each group, leading to a total of 64 subjects. We had to recruit 72 subjects to allow for 4 drop-outs per group. Details of the sample size calculation are in appendix M.

### 3.2.9 Definition of variables

Four main sources of data were used in this study, namely the Urea Breath test, the blood analyses results, the 7 day food records and the demographic questionnaire. Details of these variables are presented in the table below.

Table 3.2.1. Description, source and type of variables included in the analysis.

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dependent Variables :</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in plasma ascorbic acid and alpha tocopherol</td>
<td>Difference between pre and post supplementation levels of plasma ascorbic acid and alpha tocopherol</td>
<td>Blood sample analysis before and after supplementation</td>
</tr>
<tr>
<td><strong>Independent Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Age at the first visit</td>
<td>Questionnaire</td>
</tr>
<tr>
<td>Sex</td>
<td>Male or female</td>
<td>Questionnaire</td>
</tr>
<tr>
<td><em>H. pylori</em> Status</td>
<td>Positive or negative</td>
<td>Urea breath test</td>
</tr>
<tr>
<td>Pre-intervention intake Vit C&amp; E</td>
<td>Average daily intake of vitamin C &amp; E during the 7 days before the first blood draw</td>
<td>7 day food record</td>
</tr>
<tr>
<td>Post-intervention intake Vit C&amp; E</td>
<td>Average daily intake of vitamin C &amp; E during the 7 days before the second blood draw</td>
<td>7 day food record</td>
</tr>
<tr>
<td>% Compliance</td>
<td>Pe% Compliance= [(100 – pill coCount)/28]*100</td>
<td>Pill count of returned vitamin C and E bottles</td>
</tr>
<tr>
<td>BMI (body mass index)</td>
<td>BMI= weight (kg)/height(m)^2</td>
<td>Questionnaire</td>
</tr>
</tbody>
</table>
### Description

<table>
<thead>
<tr>
<th>Description</th>
<th>Source</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation plasma ascorbic acid, alpha tocopherol, malondialdehyde and thiols</td>
<td>Blood sample analysis before supplementation</td>
<td>Continuous</td>
</tr>
<tr>
<td>Post supplementation plasma ascorbic acid, alpha tocopherol, malondialdehyde and thiols</td>
<td>Blood sample analysis after supplementation</td>
<td>Continuous</td>
</tr>
</tbody>
</table>

### 3.2.10 Data analysis

Energy and dietary intakes were calculated as crude and energy adjusted means. The rationale for adjusting for energy, as described by Willet et al. in 1997, is to control for confounding if total energy is associated either with the disease (*H. pylori* infection) or biased reporting. In addition, adjustment for energy intake decreases extraneous variations because control for variation in total energy intake will likely reduce measurement error for specific nutrients (measurement error in the assessment of nutrient intakes is strongly associated with error in the measurement of total energy intake). For the purpose of this study, the analysis of covariance (ANCOVA) was used to estimate adjusted means. In this model, the dietary intake variable was the dependent variable, *H. pylori* infection was the fixed factor and energy consumption was the independent covariate.

Means and standard deviations for each laboratory measurement (plasma ascorbic acid, plasma alpha tocopherol, plasma MDA and plasma thiols) were calculated, in addition to the change and proportional change of these measurements after supplementation.
Change and proportional change in the plasma measurements were calculated as follows:

Change = post supplementation level – pre supplementation level

Proportional change = (Change/ Pre supplementation level) * 100

Pre and post supplementation levels of each laboratory measure in *H. pylori* negative and positive groups were compared by the paired t-test. The independent samples t-test was used to compare the *H. pylori* negative and positive groups for pre and post supplementation, change and proportional change in each measure.

Data analysis was performed using SPSS version 14.0

### 3.2.11 Methodological choices

In this section, a discussion of the methods used in the trial will be presented

**Study population: University of Toronto students and staff**

The choice of the University of Toronto population was for two main reasons. First, the students and staff at the university come from different origins, thus presenting a mix of places of birth. Second, the location of the university made its population a convenient sample for the study. The university is located near Toronto General Hospital where screening for *H. pylori* infection took place, and to Toronto Medical Laboratories where blood samples were drawn from the participants. Furthermore, there is no evidence to suggest that the effect of *H. pylori* infection on the bioavailability of vitamin E and C will be different between students and staff of the University of Toronto and other populations.
**Urea Breath test for the assessment of H. pylori infection status**

The UBT indicates current *H. pylori* status, as it detects the presence of active infection, differentiating it from other non invasive tests that diagnose both past and current infection. Gisbert et al. (2004), in their review of $^{13}$C UBT in the diagnosis of *H. pylori* infection, showed that the majority of studies that have evaluated the accuracy of this test against rapid urease test and/or histologic examination of gastric biopsies came up with a sensitivity and specificity greater than 90%. Given the relatively small sample size (compared to population studies), and the type of sample studied (healthy volunteers), the test for *H. pylori* for this study ought to be non-invasive and highly accurate therefore UBT was the method of choice.

**Dose and duration of supplementation with vitamins E and C**

In this section three main questions will be addressed: First, among all other antioxidants, why were vitamins E and C chosen for the supplementation intervention? Second, why was the period of supplementation 28 days? And last, what determined the dosage of vitamins used?

The reasons for choosing vitamins E and C for supplementation are related to their importance as antioxidants and to the increased public interest in them. The most important non enzymatic forms of antioxidant protection against free radical scavengers are the antioxidants vitamins which include vitamin C, E and Beta-carotene (Halliwell et al., 1992).

Beta-Carotene has been suggested be an important antioxidant. However, few intervention trials, in which large number of patients were given beta-carotene supplements showed negative results. In contrast to the epidemiologic observations, The Alpha-Tocopherol, Beta- Carotene Cancer Prevention (ATBC) study and the Beta-Carotene and
Retinol Efficacy Trial (CARET) indicated that supplementation of Beta-carotene and/or vitamin A in subjects having a high risk of lung cancer increased the incidence of lung cancer overall mortality (Omenn et al., 1996; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study., 1994). It has been suggested that Beta-carotene possesses pro-oxidant activity and pro-carcinogenic action in case of preexisting pre-malignant lesions (Siems et al., 2005). Given this controversy regarding the safety of beta carotene supplementation, this study was limited to vitamins E and C.

In a recent study of 1543 subjects, a survey of Natural Health Products (NHP) used in Canada ranked vitamin C and E as 2nd and 4th respectively among 28 other products used by both sexes and ranked vitamin C as the antioxidant most frequently consumed by men (Troppmann et al., 2002). The high use of these vitamins requires understanding of the factors affecting their bioavailability.

The dose of the vitamins and the duration of supplementation were chosen to be sufficient to produce an important increase in the blood levels of the vitamins but not to mask the hypothesized reducing effect of *H. pylori* infection. A review of the recent supplementation studies of vitamins E and C and of the pharmaco-kinetics of these vitamins (Viscovitch et al., 2004; Eichhorn et al., 2004), led to a choice of 400 IU of α-tocopherol and 500 mg of ascorbic acid daily over a period of twenty eight days. These dosage and duration were expected to produce a significant increase in the plasma levels of the vitamins without reaching a plateau (Eichhorn et al., 2004), so were less likely to mask the effect of *H. pylori*, if it existed. A table summarizing the main findings of the recent supplementation studies of vitamins E and C is found in appendix N. The vitamin supplements were provided by Jamiesons Laboratory. The Drug Identification Numbers (DINs) for vitamin C and E were
01994336 and 00122858 respectively. The instructions on the bottles were “take one capsule daily” for both vitamins. A recent review in the American Journal of Clinical Nutrition concluded that the clinical trial evidence shows that, for adults in the general population, vitamin E supplementation in amounts ≤ 1600 IU and vitamin C supplements ≤ 2000 mg/d are safe (Hathcock et al., 2005). The dosage of supplementation in this project is 400 IU vitamin E and 500 mg vitamin C, which is lower than the above stated tolerable levels for adults.

**Compliance**

Regimen compliance refers to maintenance of a prescribed therapeutic regimen, which in this study is the supplementation with vitamins E and C. In contrast, protocol compliance deals with schedules for clinical examinations, blood tests, or other plans of a clinical trial. In this study, regimen and protocol compliance were both assessed.

In his review, Farmer KC. (1999) discussed the advantages and disadvantages of the methods used for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. The authors classified these methods as direct or indirect for measuring patient adherence to supplementation regimens. Direct methods provide proof that the drug has been taken by the subject. These methods include: (1) detection of the drug or its metabolite in a biologic fluid, usually blood or urine; (2) detection of a biologic marker that is given with the drug; and (3) direct observation of the subject receiving the medication. The first two methods are qualitative in nature and since, in this study, we needed to measure the quantity of supplements taken, these methods were not considered. The third method was impractical in our setting, given that subjects were not institutionalized.
The indirect methods can be categorized as self reporting by the subject, pill count, use of electronic monitoring devices and prescription record review. The latter method was not feasible in this study because there was no prescription records of the subject’s supplement consumption. Although electronic monitoring devices provide precise data on regimen adherence, they are expensive and inconvenient. The choice of a method to measure regimen adherence was therefore limited to either self reporting by the subjects or pill count. Given the fact that pill count is relatively easy to use and inexpensive, this method was used in order to assess the regimen compliance in this study. Pill count may provide an overestimation of compliance if the patient is aware that a pill count is going to be conducted because subjects may remove excess doses and discard them. Therefore in this study, subjects when asked to return the bottles were not told about the pill count.

Pill counts measure regimen compliance by comparing the number of doses remaining in a container with the number of doses that should remain. It is a quantitative method of assessing compliance. Compliance was calculated by subtracting the number of pills returned from the total number of pills in the bottles. This provided an estimate of the number of pills consumed by the subject. The amount used was divided by the expected amount and multiplied by 100 to determine the percent compliance.

In this study:
Percent compliance= \[
\frac{100 – \text{pill count}}{28} \times 100
\]

*Seven-day food records for the assessment of dietary intakes of vitamins E and C*

In comparison with the 24 hour dietary recall and the food frequency questionnaires (FFQ) methods for dietary assessment, the 7-Day food records have the following advantages. First, it does not rely on memory, since the subject records the intake at the time
of consumption. Second, being an open-ended method, it can accommodate any food or food combination consumed by the subjects (Willet W., 1998). This is particularly important in a culturally diverse population such as the University of Toronto staff and students. Third and most important, compared to FFQ, The 7-day record has a higher percent agreement between quartiles of intake and biomarkers (Brunner et al., 2001). A validation study, performed in association with the UK component of the European Prospective Investigation of Cancer (EPIC), compared the 7 day food record and the food frequency questionnaire methods for dietary assessment. For this purpose, 123 individuals were asked to complete a Food Frequency questionnaire and a 7 day food record on two occasions separated by approximately 12 months. The individuals were also asked to provide 24 hour urine samples on six occasions over a 6-9 month period, covering the period over which the food frequency and the dietary records were completed. The urine was assayed for Nitrogen, potassium and sodium. The results indicated that the seven day food records provided a better estimated of average intake, as assessed by urinary measures, than did the food frequency questionnaire. The correlations between the 7 day food records estimates of intake of the three nutrients and the urinary measures were between 0.36 and 0.46, for the food frequency questionnaire the correlations varied from 0.13 to 0.22 (Day et al., 2001).
Chapter 4: Results


4.1.1 Prevalence of *H. pylori* infection in Ontario

As shown in table 4.1, the seroprevalence of *H. pylori* infection in all subjects was 29.4% (95% CI: 27.5-31.9%). Males had higher seroprevalence than females (33.0%; CI: 29.7-36.5 and 28.4%; CI: 21.4-28.5 respectively). Though the difference was not statistically significant, prevalence estimates were higher in older ages and peaked after 70 years old for both males and females (table 4.1). Weighted analysis yielded a lower estimate of the overall prevalence than the un-weighted analysis: 23.1%, 95% CI (17.7-29.5), with males still having higher prevalence (29.4%, 95% CI: 21.1-39.9) than females (14.9%, 95% CI: 10.1-21.4) (table 1). The largest difference between the weighted and un-weighted estimates was observed among females younger than 60 years (10.5% vs. 17.3%) (table 4.1).

4.1.2 Age adjusted analysis of the association of various factors with *H. pylori* infection status

The un-weighted associations of the potential risk factors with *H. pylori* infection status for each sex are presented in table 4.2. When weighted, the analysis of these associations yielded similar results for most factors. However certain differences occurred when weighted results were distorted due to small numbers in the groups studied. Weighted analyses are found in appendix O.
**Marital status**

Males and females who were married or living as married tended to have lower *H. pylori* rates than those who were single, separated, widowed or divorced (32.1% vs. 40% for males and 23.3% vs. 27.8% for females) (table 4.2), but none of these differences reached statistical significance. The age-adjusted ORs for infection also indicated a non-statistically significant lower prevalence among married subjects (table 4.2).

**Place of birth, age at immigration and ethnicity**

For males, prevalence estimates were significantly higher among subjects born outside Canada compared to those born in Canada (43.9% vs 26.6% respectively). This difference was reflected in a higher age-adjusted OR (OR: 2.2 CI: 1.6-3.0). Among non-Canadian born subjects, males who immigrated at an age older than 20 years were more likely to be infected (51.3%) compared to males who immigrated at a younger age (34.2%), as reflected in the prevalence estimates and the age-adjusted OR (OR: 1.9, 95% CI: 1.1-3.2) (Table 4.2).

For females, place of birth did not significantly affect the prevalence of the infection. Among females born outside Canada, only slightly higher prevalence estimates were observed for females who immigrated older than 20 years old (26.1% vs. 21.1% respectively) (Table 4.2).

Non-white males had higher prevalence estimates and age-adjusted OR than white males (46.4% vs., 32% and OR: 1.8, 95% CI: 1.1-3.1). For females, infection prevalence did not vary with ethnicity (Table 4.2).
**Number of siblings, education and income**

Prevalence estimates increased significantly with increasing number of siblings (p for trend <0.05 for both men and women). Prevalence was 28.8%, 36.1% and 38.6% for males with <2, 2 to 4 and more than 4 siblings respectively and 21.5%, 27.3% and 37.1% for females with <2, 2 to 4 and more than 4 siblings respectively. The age adjusted ORs were significantly higher for subjects with larger families (>4 siblings) compared to those who had 1 or no siblings (OR: 1.5, 95% CI: 1.0-2.3 and OR: 2.0, 95% CI: 1.2-3.4 for males and females respectively) (Table 4.2).

Education was negatively associated with infection (p for trend <0.05 for both sexes). Males with the highest levels of education had lower prevalence estimates compared to those with medium and low levels (25% vs. 37.9% and 35.2% respectively). For females, prevalence estimates increased with decreasing education level, p for trend <0.05 (15.4%, 25.6%, 29.3% for high, medium, and low levels respectively). These differences were reflected with significantly lower age adjusted ORs for the “high” education level (OR: 0.6; CI: 0.4-0.9 for males and OR: 0.5; CI: 0.3-0.9 for females) (Table 4.2).

For income, the number of subjects with missing data was high, so the results of this category were reported. The highest prevalence estimates were observed for males and females with the lowest income <20K compared to income 20 to 40K and >40K (Males: 45.1% vs. 41% and 30.5% respectively and Females: 33.8% vs. 23.2% and 27.3% respectively). The age adjusted ORs yielded similar findings but failed to reach significance. On the other hand, the people who did not provide information on income had the lowest prevalence estimates for both males and females (29.4% and 20.2% respectively) and
significantly lower age adjusted ORs for males (OR: 0.6; CI : 0.4-0.9) were observed (Table 4.2).

**Regular use of antacids, multivitamins, aspirin and acetaminophen**

Males who took antacids regularly had higher prevalence compared to those who did not (44% vs. 31.3%). This difference was reflected in a significant age adjusted odds ratio (OR: 1.7; CI: 1.1-2.6). Differences were substantially smaller for females (26.1 % vs 24.8%) and the association was not significant (Table 4.2)

Prevalence estimates of *H. pylori* infection among users of multivitamins were slightly lower than among non-users (28.4% vs. 35.7% for males and 23.9% vs. 26.2% for females), but were not significantly different (Table 4.2).

Among males, *H. pylori* prevalence was slightly lower among those who used aspirin regularly whereas prevalence was higher among those who used acetaminophen, though neither of these patterns was significant. Females who reported regular use of aspirin or acetaminophen had somewhat higher prevalence estimated compared to females who did not (27.8% vs. 23.4% and 30.6 vs. 23.7% respectively), though this difference was not statistically significant (Table 4.2).

**Fruit and vegetable intakes and BMI**

For males, *H. pylori* prevalence was not associated with intake of fruit. Prevalence was slightly lower in men with the highest intake of vegetables compared to others but no significant trend was observed with increasing intake. For females, lower prevalence estimates were observed with higher consumption of fruits and vegetables (28.9%, 25.7%, 21.2% for consumption of <1 servings/day, 1-2 servings per day and >2 serving/day of fruits
and 32.4%, 25.7%, 21.2%, for consumption of <1 serving/day, 1-2 servings per day and >2 servings/day of vegetables), but these trends were not significant (Table 4.2).

Among the red meat eaters, lower prevalence estimates were observed with increasing meat consumption (Males: 33.6%, 32.4%, 29.7%; Females: 26.5%, 22.4%, 20% for <1, 1-2 and >2 servings per day). However, no significant association between meat intake and *H. pylori* prevalence was observed (Table 4.2).

Infection prevalence did not vary substantially across levels of BMI (Table 4.2).

**Smoking and alcohol consumption**

Smoking was assessed by the number of years smoked and by the number of cigarettes per day. There was no significant association between smoking and the prevalence of the infection, by either measure. Males and females who smoked between 10 and 20 cigarettes per day had the highest prevalence estimates compared all other categories of smokers (Table 4.2).

For both males and females, alcohol intake was negatively associated with the prevalence of the infection (*p* of trend <0.05). Prevalence estimates decreased with increased consumption, but the patterns were substantially more marked for females (Males: 40.2%, 29.45 and 29.3%; Females: 33.2%, 19.3%, 9.1% for never, ≤10 servings per week and > 10 servings per week respectively). Subjects consuming alcohol had significantly lower age adjusted odds of being infected compared to those who never drank (Table 4.2).

**Incidence of polyps, inflammatory bowel disease (IBD), diabetes and cancer.**

Subjects who reported having polyps had slightly higher estimates of *H. pylori* infection prevalence compared to those who did not (Males: 36% vs. 32.8% respectively; females: 35.7% vs 23.9% respectively). In contrast, prevalence of infection was lower among
subjects who had IBD compared to those without the disease (Males: 23.5% vs. 33% respectively; females: 14.3% vs. 26.3%). However none of these differences reached statistical significance (Table 4.2).

Prevalence of the infection was slightly lower among diabetic men (29.1% vs 33.4%). This observation was reversed for women, who had a higher prevalence in diabetics (35.3% vs. 24.2%). These differences were not statistically significant.

No difference in prevalence estimates was observed between subjects who had cancer and those who did not.

### 4.1.3 Multivariate analysis of the association of various factors with *H. pylori* infection status

The final multivariate model found to be the best predictor of *H. pylori* infection included age, sex, age at immigration to Canada, number of siblings, alcohol intake and two interaction terms (sex*age and sex*age at immigration) (Table 4.3). The Hosmer and Lemeshow test of the goodness of fit of this model yielded a p value of 0.92, indicating the model fitted well. Infection was positively associated with age of migration to Canada. Age greater than 20 years at migration was associated with higher odds of being infected compared to those born in Canada (OR: 2.9, 95% CI: 1.9-4.3). The odds of infection were also increased among those who immigrated younger than 20 years (OR: 1.6, 95% CI: 1.0-2.5). Infection was positively associated with number of siblings with the highest odds observed with more than 4 siblings (OR: 1.7, 95% CI: 1.2-2.4). The risk of infection was negatively associated with alcohol consumption, subjects consuming alcohol had significantly lower odds of being infected compared to subjects who never drank (Table 4.3). Smoking, fruit and vegetable intake and incidence of diabetes were each entered separately in
the model, as they have been shown to be associated with *H. pylori* seropositivity in other studies, but none of these variables had an effect and so were not retained.

Since both interaction terms which included the sex variable were significant, a separate multivariate model was created for men and women.

In the final model for males (Table 4.4), place of birth and age at immigration, number of siblings, intake of antacids and alcohol consumption were each significantly associated with the infection status. Males who immigrated to Canada older than 20 years old were at increased risk of infection compared to the males who are born in Canada (OR: 2.9; CI: 1.9-4.2). The larger the family the male subject belonged to the higher the odds of the infection he had (OR: 1.4, CI: 1.2-2.2 and OR: 1.6; CI: 1.1-2.4 for males having 2-4 and >4 siblings respectively) (p for trend <0.05). Regular use of antacids was significantly associated with a positive *H. pylori* infection status (OR: 1.6; CI: 1.1-2.5). Prevalence of the infection was lower among males who drank compared to those who never drank (Table 4.4).

The final model for females included age, number of siblings and alcohol intake as significantly associated with of *H. pylori* infection status (table 4.5). Females older than 70 years were at a higher risk of infection compared to those younger than 60 years (OR: 2.8; CI: 1.6-4.7). The risk of the infection increased with the number of siblings (p for trend <0.05) and was significantly higher among females belonging to families with more than 4 siblings when compared to those from families with less than 2 siblings (OR: 1.9; CI: 1.1 - 1.3). Lower prevalence estimates were observed with increasing consumption of alcohol. Females who drank 10 or less servings of alcohol were at a lower risk of the infection compared to those who never drink (OR: 0.5; CI: 0.3-0.9), and risk was lower still among those who consumed more alcohol (OR=0.2; CI: 0.0-1.1). Despite its small size, this OR was
not significant, probably because of the small number of women who consumed this amount of alcohol (22) (Table 4.5).
Table 4.1.1  Frequency distribution, unweighted and weighted % *H. pylori* seropositivity, and odds ratio estimates and their 95% confidence intervals for males and females by various age groups in the control sample of the OFCCR.

<table>
<thead>
<tr>
<th>Age</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>%positive (95% CI)</td>
</tr>
<tr>
<td>&lt;60 yrs</td>
<td>218 29.4 (23.7-35.8)</td>
<td>27.7 (17.6-41.0)</td>
</tr>
<tr>
<td>60-70 yrs</td>
<td>321 32.1 (27.2-37.4)</td>
<td>31.96 (27.0-37.4)</td>
</tr>
<tr>
<td>&gt;70 yrs</td>
<td>194 38.7 (32.0-45.7)</td>
<td>38.7 (32.0-45.8)</td>
</tr>
<tr>
<td>Total</td>
<td>733 33.0 (29.7-36.5)</td>
<td>29.4 (21.1-39.3)</td>
</tr>
</tbody>
</table>

† Weighted values presented

Average age ± standard deviation (mean) were 64.7 ± 8.0 and 63.2 ± 9.4 years in males and females respectively.
Table 4.1.2 Frequency distribution, % *H. pylori* seropositivity, age adjusted odds ratio estimates and their 95% confidence intervals in males and females of the control sample of the OFCCR.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>%positive (95% CI)</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not married</td>
<td>90</td>
<td>40.0 (30.4-50.4)</td>
</tr>
<tr>
<td>Married</td>
<td>633</td>
<td>32.1 (28.5-35.8)</td>
</tr>
<tr>
<td><strong>Place of Birth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>462</td>
<td>26.6 (22.8-30.8)</td>
</tr>
<tr>
<td>Other</td>
<td>271</td>
<td>43.9 (38.1-49.9)</td>
</tr>
<tr>
<td><strong>Age of immigration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 yrs</td>
<td>111</td>
<td>34.2 (26-43.5)</td>
</tr>
<tr>
<td>&gt;20 yrs</td>
<td>152</td>
<td>51.3 (43.4-59.2)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>676</td>
<td>32.0 (28.5-35.56)</td>
</tr>
<tr>
<td>Non white</td>
<td>56</td>
<td>46.4 (33.9-59.5)</td>
</tr>
<tr>
<td><strong>Number of Siblings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>316</td>
<td>28.8 (24.1-34)</td>
</tr>
<tr>
<td>2-4</td>
<td>180</td>
<td>36.1 (29.4-43.4)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>158</td>
<td>38.6 (31.3-46.4)</td>
</tr>
<tr>
<td>Missing</td>
<td>79</td>
<td>31.6 (22.3-42.7)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>281</td>
<td>35.2 (29.8-41.0)</td>
</tr>
<tr>
<td>Middle</td>
<td>232</td>
<td>37.9 (31.9-44.4)</td>
</tr>
<tr>
<td>High</td>
<td>212</td>
<td>25.0 (19.6-31.3)</td>
</tr>
</tbody>
</table>
Table 4.1.2 continued.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>%positive (95% CI)</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 K</td>
<td>185</td>
<td>42.2 (35.2-49.4)</td>
</tr>
<tr>
<td>20-40K</td>
<td>230</td>
<td>30.9 (25.2-37.1)</td>
</tr>
<tr>
<td>&gt;40K</td>
<td>19</td>
<td>26.3 (11.4-49.8)</td>
</tr>
<tr>
<td>Missing</td>
<td>299</td>
<td>29.4 (24.5-34.9)</td>
</tr>
<tr>
<td>Regular use of Antacids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>620</td>
<td>31.3 (27.7-35.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>100</td>
<td>44.0 (34.6-53.9)</td>
</tr>
<tr>
<td>Regular use of Multivitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>445</td>
<td>35.7 (31.4-40.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>278</td>
<td>28.4 (23.4-34)</td>
</tr>
<tr>
<td>Regular use of Aspirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>393</td>
<td>33.6 (29.1-38.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>327</td>
<td>31.5 (26.7-36.7)</td>
</tr>
<tr>
<td>Regular use of Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>638</td>
<td>32.0 (28.4-35.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>40.7 (30.6-51.7)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>173</td>
<td>32.0 (25.4-39.3)</td>
</tr>
<tr>
<td>25-30</td>
<td>371</td>
<td>33.7 (29.0-38.7)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>163</td>
<td>33.7 (26.9-41.3)</td>
</tr>
<tr>
<td>Fruit Intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1/day</td>
<td>190</td>
<td>31.3 (24.9-38)</td>
</tr>
<tr>
<td>1-2/day</td>
<td>424</td>
<td>33.3 (28.9-37.9)</td>
</tr>
<tr>
<td>&gt;2/day</td>
<td>90</td>
<td>33.3 (24.3-43.7)</td>
</tr>
</tbody>
</table>
Table 4.1.2 continued.

<table>
<thead>
<tr>
<th></th>
<th>Total no.</th>
<th>%positive (95% CI)</th>
<th>OR (95% CI)</th>
<th>Total no.</th>
<th>%positive (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1/day</td>
<td>116</td>
<td>34.5 (26.4-43.6)</td>
<td>1.0</td>
<td>34</td>
<td>32.4 (18.9-49.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>1-2/day</td>
<td>478</td>
<td>33.1 (29-37.4)</td>
<td>0.9</td>
<td>298</td>
<td>25.8 (21.2-31.1)</td>
<td>0.7</td>
</tr>
<tr>
<td>&gt;2/day</td>
<td>122</td>
<td>30.3 (22.8-39.1)</td>
<td>0.8</td>
<td>232</td>
<td>22.0 (17.1-27.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>Red Meat Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 servings/week</td>
<td>252</td>
<td>36.5 (30.8-42.6)</td>
<td>1.0 (0.6-1.2)</td>
<td>241</td>
<td>28.6 (23.3-34.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>3-5 servings/week</td>
<td>309</td>
<td>31.7 (26.8-37.1)</td>
<td>0.8 (0.6-1.2)</td>
<td>224</td>
<td>22.3 (17.3-28.3)</td>
<td>0.7 (0.5-1.1)</td>
</tr>
<tr>
<td>&gt;5 servings/week</td>
<td>158</td>
<td>29.7 (23.1-37.3)</td>
<td>0.7 (0.5-1.1)</td>
<td>98</td>
<td>21.4 (14.4-30.7)</td>
<td>0.7 (0.4-1.2)</td>
</tr>
<tr>
<td>Smoking yrs smoking*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>259</td>
<td>32.4 (27.4-39)</td>
<td>1.0</td>
<td>293</td>
<td>24.6 (20-29.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;10 yrs</td>
<td>62</td>
<td>29.0 (19.1-41.5)</td>
<td>0.8 (0.5-1.6)</td>
<td>53</td>
<td>17.0 (9.1-29.6)</td>
<td>0.7</td>
</tr>
<tr>
<td>10-25</td>
<td>164</td>
<td>34.1 (27.3-41.7)</td>
<td>1.1</td>
<td>70</td>
<td>25.7 (16.8-37.2)</td>
<td>1.2</td>
</tr>
<tr>
<td>25-40</td>
<td>134</td>
<td>35.1 (27.5-43.5)</td>
<td>1.1</td>
<td>83</td>
<td>26.5 (18.1-37)</td>
<td>1.3</td>
</tr>
<tr>
<td>&gt;40</td>
<td>68</td>
<td>30.9 (21.1-42.8)</td>
<td>0.8 (0.5-1.5)</td>
<td>50</td>
<td>36.0 (23.5-50.1)</td>
<td>1.4</td>
</tr>
<tr>
<td>No. cigarettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>259</td>
<td>32.4 (27.4-38.4)</td>
<td>1.0</td>
<td>293</td>
<td>24.6 (20-29.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;10/day</td>
<td>130</td>
<td>32.3 (24.8-40.8)</td>
<td>0.9 (0.6-1.5)</td>
<td>113</td>
<td>18.6 (12.4-26.9)</td>
<td>0.7</td>
</tr>
<tr>
<td>10-20/day</td>
<td>189</td>
<td>36.0 (29.4-43.1)</td>
<td>1.1</td>
<td>88</td>
<td>30.7 (21.9-41.1)</td>
<td>1.4</td>
</tr>
<tr>
<td>&gt;20/day</td>
<td>124</td>
<td>29.8 (22.4-38.5)</td>
<td>0.9 (0.5-1.4)</td>
<td>57</td>
<td>28.1 (17.9-41.1)</td>
<td>1.4</td>
</tr>
<tr>
<td>Alcohol Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>127</td>
<td>40.2 (32-48.9)</td>
<td>1.0</td>
<td>244</td>
<td>33.2 (27.6-39.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>≤10d/week</td>
<td>286</td>
<td>29.4 (24.4-34.9)</td>
<td>0.6 (0.4-1.0)</td>
<td>145</td>
<td>19.3 (13.7-26.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>&gt;10d/week</td>
<td>167</td>
<td>29.3 (22.9-36.7)</td>
<td>0.6 (0.4-1.0)</td>
<td>22</td>
<td>9.1 (2.3-30)</td>
<td>0.2</td>
</tr>
<tr>
<td>Polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>629</td>
<td>32.8 (29.2-36.5)</td>
<td>1.1 (0.7-1.8)</td>
<td>497</td>
<td>23.9 (20.4-27.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>89</td>
<td>36.0 (26.7-46.4)</td>
<td>1.6 (0.9-3)</td>
<td>56</td>
<td>35.7 (24.3-49)</td>
<td>1.6 (0.9-3)</td>
</tr>
</tbody>
</table>
Table 4.1.2 continued.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>%positive (95% CI)</td>
</tr>
<tr>
<td><strong>IBD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>693</td>
<td>33.0 (29.6-36.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>23.5 (9.1-48.6)</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>637</td>
<td>33.4 (30-37.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>86</td>
<td>29.1 (20.4-39.5)</td>
</tr>
<tr>
<td><strong>Incidence of any cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>608</td>
<td>33.2 (29.6-37.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>114</td>
<td>30.7 (22.9-39.8)</td>
</tr>
</tbody>
</table>
Table 4.1.3 Logistic regression model with odds ratio estimates and 95% confidence intervals for *H. pylori* seropositivity in the control sample of the OFCCR (males and females).

<table>
<thead>
<tr>
<th></th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>1</td>
</tr>
<tr>
<td>60-70</td>
<td>1.1 (0.8-1.7)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1.4 (0.9-2.1)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>0.6 (0.3-1.0)</td>
</tr>
<tr>
<td><strong>Age at immigration to Canada</strong></td>
<td></td>
</tr>
<tr>
<td>Born in Canada</td>
<td>1</td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>1.6 (1.0-2.5)</td>
</tr>
<tr>
<td>&gt;20 years</td>
<td>2.9 (1.9-4.3)</td>
</tr>
<tr>
<td><strong>Number of Siblings</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>2-4</td>
<td>1.4 (1.0-1.9)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>1.7 (1.2-2.4)</td>
</tr>
<tr>
<td><strong>Alcohol Intake</strong></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
</tr>
<tr>
<td>&lt;10d/week</td>
<td>0.6 (0.4-0.8)</td>
</tr>
<tr>
<td>&gt;10d/week</td>
<td>0.5 (0.3-0.8)</td>
</tr>
<tr>
<td><strong>Interaction Sex*age</strong></td>
<td></td>
</tr>
<tr>
<td>Sex(Females)*age(60-70)</td>
<td>1.3 (0.7-2.4)</td>
</tr>
<tr>
<td>Sex(Females)*age(&gt;70)</td>
<td>2.0 (1.0-4.0)</td>
</tr>
<tr>
<td>*<em>Interaction Sex <em>age at immigration</em></em></td>
<td></td>
</tr>
<tr>
<td>Sex(Females)*immigration(&lt;20yrs)</td>
<td>0.4 (0.2-1.0)</td>
</tr>
<tr>
<td>Sex(Females)*immigration(&gt;20yrs)</td>
<td>0.3 (0.2-0.7)</td>
</tr>
</tbody>
</table>
Table 4.1.4 Logistic regression model with odds ratio estimates and 95% confidence intervals for *H. pylori* seropositivity in the control sample of the OFCCR (males).

<table>
<thead>
<tr>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>&lt;60</td>
</tr>
<tr>
<td>60-70</td>
</tr>
<tr>
<td>&gt;70</td>
</tr>
<tr>
<td><strong>Age at immigration to Canada</strong></td>
</tr>
<tr>
<td>Born in Canada</td>
</tr>
<tr>
<td>&lt;20 years of age</td>
</tr>
<tr>
<td>&gt;20 years of age</td>
</tr>
<tr>
<td><strong>Number of Siblings</strong></td>
</tr>
<tr>
<td>&lt;2</td>
</tr>
<tr>
<td>2-4</td>
</tr>
<tr>
<td>&gt;4</td>
</tr>
<tr>
<td><strong>Intake of antiacids</strong></td>
</tr>
<tr>
<td>Do not take</td>
</tr>
<tr>
<td>Take</td>
</tr>
<tr>
<td><strong>Alcohol Intake</strong></td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td>&lt;10d/week</td>
</tr>
<tr>
<td>&gt;10d/week</td>
</tr>
</tbody>
</table>

Table 4.1.5 Logistic regression model with odds ratio estimates and 95% confidence intervals for *H. pylori* seropositivity in control sample of the OFCCR (females).

<table>
<thead>
<tr>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>&lt;60</td>
</tr>
<tr>
<td>60-70</td>
</tr>
<tr>
<td>&gt;70</td>
</tr>
<tr>
<td><strong>Number of Siblings</strong></td>
</tr>
<tr>
<td>&lt;2</td>
</tr>
<tr>
<td>2-4</td>
</tr>
<tr>
<td>&gt;4</td>
</tr>
<tr>
<td><strong>Alcohol Intake</strong></td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td>&lt;10d/week</td>
</tr>
<tr>
<td>&gt;10d/week</td>
</tr>
</tbody>
</table>
4.2 Project II: Effect of \textit{H. pylori} infection on the bioavailability of vitamins E and C

Screening volunteers for this study took place between August 2006 and June 2007. Recruitment of \textit{H. pylori} negative and positive subjects happened simultaneously during this period. Below is a schematic representation of the number of subjects during the screening/recruitment phase.

164 subjects tested for \textit{H. pylori} infection

132 negatives (80.0%)  32 positives (19.5%)

40 negatives recruited  32 positives recruited

34 negatives finished  (6 dropped out, 15%)  27 positives finished  (5 dropped out, 16%)

Among 164 volunteers screened for \textit{H. pylori} infection, 32 were \textit{H. pylori} positive. All positive subjects were recruited in the study. Whenever a positive subject was identified and recruited, another negative subject, who was screened within one week time interval, was recruited. Overall 72 volunteers were recruited, 40 \textit{H. pylori} negative and 32 \textit{H. pylori} positive subjects. Sixty one participants completed the study (34 negative and 27 positive subjects). Failure to adhere to the study protocol was a main reason for drop out (4 \textit{H. pylori} negative and 5 \textit{H. pylori} positive subjects did not come to
the last visit, therefore were dropped out). The remaining drop-outs were due to moving
out of Toronto. There were no important differences in mean age or sex distribution
between subjects who dropped out and those who completed the study (drop outs, age:
25.82± 7.4 yrs with 40.8% males, participants who completed the study, age: 27.28±7.2
yrs with 44.3 % males).

Originally, 32 subjects in each group were needed in order to achieve a power of
80% to detect an effect size of half the standard deviation of the outcome measure. The
availability of 34 H. pylori negative and 27 H. pylori positive subjects allowed, at the
same power, the detection of 0.57 of the standard deviation.

4.2.1 Demographic characteristics of study participants

The mean age for all participants was 27.91 ± 13.97 yrs, with H. pylori negative
subjects being slightly younger than the positives (25.56 ± 6.56 vs. 29.41 ± 7.58 yrs
respectively). The percentage of males within the H. pylori positive group was
significantly higher than that within the negative group (60% vs. 32% respectively) (table
4.2.1).

Table 4.2.1. Demographic characteristics of H. pylori negative and positive subjects.

<table>
<thead>
<tr>
<th></th>
<th>H. pylori negatives (n=34)</th>
<th>H. pylori positives (n=27)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD)</td>
<td>25.56±6.56 yrs</td>
<td>29.41±7.58 yrs</td>
<td>p=0.22</td>
</tr>
<tr>
<td>Sex</td>
<td>Males: 11 (32%)</td>
<td>Males: 16 (60%)</td>
<td>p=0.04</td>
</tr>
<tr>
<td></td>
<td>Females: 23 (68%)</td>
<td>Females: 11 (40%)</td>
<td></td>
</tr>
<tr>
<td>BMI (mean±SD)</td>
<td>23.42±4.78 kg/m²</td>
<td>24.19± 3.46 kg/m²</td>
<td>p=0.20</td>
</tr>
<tr>
<td>Marital status</td>
<td>Single: 26 (76%)</td>
<td>Single: 18 (67%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Married: 8 (24%)</td>
<td>Married: 8 (33%)</td>
<td>p=0.20</td>
</tr>
</tbody>
</table>

* p-values for age and BMI were derived from independent samples t-test, and from chi-
square test for the sex and marital status distributions.
4.2.2 Compliance to supplementation with vitamin E and C

Compliance to the supplementation protocol of vitamin C and vitamin E was derived from the pill count of returned pills of these vitamins supplements:

\[
\text{Percent compliance} = \left[ \frac{100 - \text{pill count}}{28} \right] \times 100
\]

Compliance data was missing for two subjects who failed to bring back their leftover vitamins (one subject forgot both bottles of vitamins while the other subject did not bring back his vitamin E leftover pills). For vitamin E and C, percent compliance was similar between \textit{H. pylori} negative and positive subjects. Over 95% compliance was achieved for both vitamins (table 4.2.2).

Table 4.2.2 Compliance (%) for vitamin E and C supplementation in \textit{H. pylori} negative and positive subjects†

<table>
<thead>
<tr>
<th></th>
<th>\textit{H. pylori} negative</th>
<th>\textit{H. pylori} positive</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (n=32)</td>
<td>(n=32) 96.43 ± 11.55</td>
<td>(n=27) 97.88 ± 6.66</td>
<td>0.43</td>
</tr>
<tr>
<td>Vitamin C (n=33)</td>
<td>(n=33) 96.86 ± 7.67</td>
<td>(n=27) 96.56 ± 8.72</td>
<td>0.82</td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SDs
* p-values were derived from independent samples t-tests between \textit{H. pylori} negative and positive groups.

4.2.3 Dietary intakes.

Energy and nutrient intake data was obtained from the 7-day food records completed by subjects before the first and second blood tests (week 0 and week 4 of the study). Average intakes of the 7 day food records for week 0 and week 4 are calculated for \textit{H. pylori} negative and positive subjects. Two \textit{H. pylori} negative subjects failed to bring the food records for week 0 and week 4. One \textit{H. pylori} positive subject did not hand in the food records for week 0. Dietary intakes of \textit{H. pylori} negative and positive
subjects were compared using the independent samples t-tests for comparison of means (SPSS, 14.1).

*H. pylori* negative subjects reported consuming slightly more energy (Kcal) than the *H. pylori* positives during both week 0 and 4 (week 0: 2164.87± 500.06 vs. 1928.97± 462.27; p=0.10 and week 4: 2043±441.88 vs. 1902.02±563.11, p=0.24 respectively) (Table 4.2.3). In order to adjust for this difference in energy consumption, energy adjusted means were calculated for all nutrients. Un-adjusted dietary intake data is found in appendix P.

Adjusted means of intake of most nutrients were similar between the two groups both in week 0 and week 4 with the exception of fiber intake, which remained higher among the *H. pylori* negative subjects (week 0: 20.67±6.33 vs. 16.59±6.26; p=0.02; week 4: 19.055±6.17 vs. 15.56±6.34; p=0.01) (Table 4.2.3).

Energy adjusted intakes of vitamin C and E were similar between *H. pylori* negative and positive groups at week 0 and week 4 (at week 0, vitamin C (mg): 109.25±51.37 vs 91.68±50.77 respectively; p=0.17, vitamin E (mg): 5.01±1.81 vs. 4.571±1.79 respectively; p=0.35; week 4, vitamin C (mg): 105.12±58.87 vs. 97.45±60.48 respectively; p=0.4, vitamin E: 4.55±1.63 vs. 4.21±1.67; p=0.45 respectively) (Table 4.2.3).

Comparison of dietary intakes, by a paired t-test, between week 0 and week 4 in *H. pylori* negative and *H. pylori* positive groups showed that the consumption of energy and nutrients did not change over the course of the study in both groups (p>0.05). Means and standard deviations of energy consumption and energy adjusted nutrient intakes are presented in table 4.2.3.
Table 4.2.3. Energy and energy adjusted intakes during week 0 and week 4 of the study for *H. pylori* negative and positive subjects†.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>H. pylori</em> –ve</td>
<td><em>H. pylori</em> +ve</td>
</tr>
<tr>
<td><strong>Energy (Kcal)</strong></td>
<td>2164.87 ± 500.06</td>
<td>1928.97 ± 462.27</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>266.32 ± 38.42</td>
<td>270.24 ± 37.97</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>83.94 ± 15.49</td>
<td>78.94 ± 15.31</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>76.12 ± 14.01</td>
<td>76.02 ± 13.84</td>
</tr>
<tr>
<td><strong>Fiber (g)</strong></td>
<td>20.67 ± 6.33</td>
<td>16.59 ± 6.26</td>
</tr>
<tr>
<td><strong>Thiamin (mg)</strong></td>
<td>1.48 ± 0.40</td>
<td>1.49 ± 0.4</td>
</tr>
<tr>
<td><strong>Riboflavin (mg)</strong></td>
<td>1.60 ± 0.39</td>
<td>1.56 ± 0.39</td>
</tr>
<tr>
<td><strong>Niacin (mg)</strong></td>
<td>20.21 ± 5.87</td>
<td>19.35 ± 5.8</td>
</tr>
<tr>
<td><strong>Vitamin B6 (mg)</strong></td>
<td>1.71 ± 0.65</td>
<td>1.42 ± 0.64</td>
</tr>
<tr>
<td><strong>Vitamin B12 (µg)</strong></td>
<td>3.64 ± 2.19</td>
<td>3.93 ± 2.16</td>
</tr>
<tr>
<td><strong>Folate (µg)</strong></td>
<td>383.93 ± 241.77</td>
<td>431.01 ± 238.95</td>
</tr>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td>109.25 ± 51.37</td>
<td>91.68 ± 50.77</td>
</tr>
<tr>
<td><strong>Vitamin D (mg)</strong></td>
<td>2.58 ± 1.71</td>
<td>2.05 ± 1.69</td>
</tr>
<tr>
<td><strong>Vitamin A (µg)</strong></td>
<td>1912.78 ± 1061.69</td>
<td>1792.26 ± 1049.29</td>
</tr>
<tr>
<td><strong>Vitamin E (mg)</strong></td>
<td>5.01 ± 1.81</td>
<td>4.57 ± 1.79</td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td>767.21 ± 185.80</td>
<td>679.82 ± 183.63</td>
</tr>
<tr>
<td><strong>Iron (mg)</strong></td>
<td>16.23 ± 4.36</td>
<td>14.69 ± 4.31</td>
</tr>
<tr>
<td><strong>Magnesium (mg)</strong></td>
<td>252.39 ± 68.10</td>
<td>225.02 ± 67.31</td>
</tr>
<tr>
<td><strong>Potassium (mg)</strong></td>
<td>2418.30 ± 572.73</td>
<td>2272.18 ± 566.04</td>
</tr>
<tr>
<td><strong>Sodium (mg)</strong></td>
<td>3083.46 ± 706.63</td>
<td>2815.69 ± 698.38</td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SDs

*p-value derived from independent samples t-test between *H. pylori* negative and positive groups.
4.2.3 Pre and post supplementation plasma ascorbic acid, alpha tocopherol, malondialdehyde and thiols of *H. pylori* negative and positive subjects.

In this section, results of blood samples analyses of plasma ascorbic acid, alpha tocopherol, malondialdehyde (MDA) and thiols are presented. Identification and exclusion of outliers were based on extreme values in the distribution of proportional change for various measurements in this study. An outlier was defined as a value falling outside the range of the mean ± 3 SDs.

4.2.3.1 Plasma ascorbic acid (umol/l).

In total, 120 out of 122 samples were available for plasma ascorbic acid assessment. Two samples were not analyzed and possibly were lost during the transport of the samples from Toronto General Hospital (where the samples were stored) to the laboratory in the Medical Sciences building at the University of Toronto (where the analysis took place). Another 2 samples had pre-supplementation measures below zero (due to mishandling of the samples during the analysis); therefore their pre and post results were discarded. One subject had 1531% proportional change in plasma ascorbic acid (fig. 4.2.1). The mean and standard deviation of the proportional change of all subjects was 79.31± 209.37. This value is greater than the mean ± 3 SDs and therefore was excluded from the analysis. This extreme value is due to a very low pre supplementation level (6.67 umol/l) despite a normal pre supplementation intake of vitamin C (100.54 mg/day).
Post supplementation plasma ascorbic acid levels were significantly higher than pre supplementation levels in both groups (*H. pylori* negative: 64.46±16.89 vs. 50.49±18.00; *H. pylori* positive: 71.17±23.27 vs. 50.30±22.04 respectively). Comparison of the *H. pylori* negative and positive groups showed that at baseline plasma ascorbic acid levels were comparable (50.49±18.00 and 50.30±22.04 respectively). Post supplementation levels were slightly lower in the *H. pylori* negative group compared to the positive group (64.46±16.89 vs 71.17±23.27 respectively). However, this difference did not reach statistical significance. Given the large standard deviations of the plasma measures of plasma ascorbic acid in both groups, a log transformation was undertaken for these variables before running the comparison of means. A constant (K=50) was added to bring the change in plasma ascorbic acid to above zero in order to run the log transformation.
Change and proportional change were lower, but not significantly, among *H. pylori* negative group compared to *H. pylori* positive (Change: 13.97±16.86 vs. 20.87±27.66 respectively; proportional change: 44.32±76.91 vs. 66.91±82.34 respectively) (Table 4.2.4).

Table 4.2.4 Pre and post supplementation, change and proportional change in plasma ascorbic acid in *H. pylori* –ve and positive subjects†.

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> –ve (n=33)</th>
<th><em>H. pylori</em> +ve (n=24)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation (umol/l)</td>
<td>50.49±18.00</td>
<td>50.30±22.04</td>
<td>0.75</td>
</tr>
<tr>
<td>Post supplementation (umol/l)</td>
<td>64.46±16.89</td>
<td>71.17±23.27</td>
<td>0.39</td>
</tr>
<tr>
<td>p-value**</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Change (umol/l)</td>
<td>13.97±16.86</td>
<td>20.87±27.66</td>
<td>0.76</td>
</tr>
<tr>
<td>Proportional change (%)</td>
<td>44.32±76.91</td>
<td>66.91±82.34</td>
<td>0.60</td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from independent t test for comparison of means between *H. pylori* negative and positive group (log transformed data)
** p-value derived from paired t test for comparison of pre and post supplementation plasma levels (log transformed data)

Figure 4.2.2 illustrates pre and post supplementation plasma ascorbic acid concentration for each subject in the *H. pylori* negative and *H. pylori* positive groups. Seven subjects (20%) in the *H. pylori* negative group and 5 subjects (20%) in the *H. pylori* positive had negative change in their plasma levels (fig 4.2.2). All of these subjects, except for one, belonged to the third and fourth quartiles of the distribution of plasma ascorbic acid at baseline.
Figure 4.2.2. Pre and post supplementation levels of plasma ascorbic acid in *H. pylori* negative and positive subjects.

![Graph showing plasma ascorbic acid levels](image)

Analysis of pre and post supplementation, the change, and proportional change of plasma ascorbic acid by quartiles of pre supplementation levels distribution in *H. pylori* negative and positive groups is presented in table (4.2.5). Change and proportional change were lower in the 3rd and 4th quartiles compared to the 1st and 2nd quartiles in both groups. Significant differences between *H. pylori* negative and positive groups were observed in the 3rd quartile. Despite the fact that *H. pylori* negative group had higher pre supplementation plasma ascorbic acid levels than *H. pylori* positive group (58.39±3.46 vs. 52.56±4.59 respectively; p <0.05), this group had significantly lower post supplementation, change and proportional change (post supplementation: 60.51±11.94 vs. 77.61±14.08; change: 2.12±12.97 vs. 25.06±16.72; proportional change: 4.11±22.17 vs 49.41±34.58 respectively).
Fig 4.2.3 illustrates pre and post plasma ascorbic acid concentration by quartiles of plasma ascorbic acid at baseline in *H. pylori* negative and positive subjects.

Table 4.2.5 Pre and post supplementation levels, change and proportional change of plasma ascorbic acid by quartiles of pre supplementation levels distribution in *H. pylori* negative and positive subjects†.

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Pre supplementation (umol/l)</th>
<th>Post supplementation (umol/l)</th>
<th>p-value*</th>
<th>Change (umol/l)</th>
<th>Proportional Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (8)</td>
<td>28.01 ± 6.70</td>
<td>56.72±12.37</td>
<td>0.002</td>
<td>28.71±16.16</td>
<td>123.78±120.72</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>24.50±8.13</td>
<td>53.93±18.07</td>
<td>0.01</td>
<td>29.43±18.10</td>
<td>141.85±99.00</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.41</td>
<td>0.64</td>
<td>0.89</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (9)</td>
<td>43.47±4.54</td>
<td>59.18±11.50</td>
<td>0.004</td>
<td>15.71±12.60</td>
<td>37.69±32.55</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>43.03±3.85</td>
<td>77.99±34.78</td>
<td>0.02</td>
<td>34.96±34.05</td>
<td>80.99±76.21</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.87</td>
<td>0.25</td>
<td>0.22</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>3rd quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (8)</td>
<td>58.39±3.46</td>
<td>60.51±11.94</td>
<td>0.81</td>
<td>2.12±12.97</td>
<td>4.11±22.17</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>52.56±4.59</td>
<td>77.61±14.08</td>
<td>0.01</td>
<td>25.06±16.72</td>
<td>49.41±34.58</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.03</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>4th quartile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (8)</td>
<td>72.97±10.59</td>
<td>82.09±19.51</td>
<td>0.18</td>
<td>9.12±15.51</td>
<td>12.54±22.14</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>81.12±11.15</td>
<td>75.13±16.60</td>
<td>0.53</td>
<td>-5.99±23.23</td>
<td>-4.62±30.04</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.20</td>
<td>0.52</td>
<td>0.24</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from paired t test comparing pre and post supplementation levels of plasma ascorbic acid (log transformed data).
** p-value derived from independent t test comparing *H. pylori* negative and positive groups (log transformed data).
Fig 4.2.3. Pre and post plasma ascorbic acid concentration by quartiles of plasma ascorbic acid at baseline in *H. pylori* negative and positive subjects.
4.2.3.2 Plasma alpha tocopherol (umol/l)

All blood samples collected (n=122) were analyzed for alpha tocopherol. Proportional change values for all subjects were within 3 SDs of the mean (mean ± SD: 72.37 ± 54.41 umol/l) (fig 4.2.4), therefore none was excluded.

Fig 4.2.4. Proportional change (%) in plasma alpha tocopherol in all subjects.

Means and standard deviations of plasma alpha tocopherol before and after supplementation for *H. pylori* negative and positive groups are presented in table 4.2.6. Post supplementation plasma alpha tocopherol levels were significantly higher than pre supplementation levels in *H. pylori* negative and positive groups (*H. pylori* negative: 37.35±8.35 vs. 21.83±6.76 respectively; *H. pylori* positive: 38.27±19.04 vs. 23.80±5.66 respectively). Comparison of *H. pylori* negative and positive groups showed no significant differences between the two groups in pre supplementation, post supplementation and change in plasma alpha tocopherol levels. Similar to change and proportional change in plasma
ascorbic acid, a log transformation was warranted in the case of plasma alpha tocopherol because of the large standard deviation of these variables. Constants were added to bring the change (+20) and proportional change (+100) to above zero levels. Proportional change in the *H. pylori* negative group was higher than the *H. pylori* positive group (83.15±54.49 vs. 58.79±52.16); however this substantial difference did not reach statistical significance at a conventional 5% levels because of the small sample size. When log transformed, there was little difference between the proportional change in plasma alpha tocopherol between the *H. pylori* negative and positive group (5.16±0.32 vs. 4.97±0.56 respectively, p=0.13)

Table 4.2.6 Pre and post supplementation, change and proportional change in plasma alpha tocopherol in *H. pylori* –ve and positive subjects†.

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> –ve (n=34)</th>
<th><em>H. pylori</em> +ve (n=27)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(umol/l)</td>
<td>21.83±6.76</td>
<td>23.80±5.66</td>
<td>0.14</td>
</tr>
<tr>
<td>Post supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(umol/l)</td>
<td>37.35±8.35</td>
<td>38.27±19.04</td>
<td>0.50</td>
</tr>
<tr>
<td>p-value**</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Change (umol/l)</td>
<td>15.52±9.40</td>
<td>14.47±15.77</td>
<td>0.39</td>
</tr>
<tr>
<td>Proportional change (%)</td>
<td>83.15±54.49</td>
<td>58.79±52.16</td>
<td>0.13</td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from independent t test for comparison of means between *H. pylori* negative and positive group (log transformed data).
** p-value derived from paired t test for comparison of pre and post supplementation plasma levels (log transformed data).

Pre and post supplementation levels of plasma alpha tocopherol for each subject in the *H. pylori* negative and positive groups are illustrated in fig. 4.2.5. In a total of 5 subjects, post supplementation plasma alpha tocopherol levels were lower than pre supplementation levels (3 subjects (8.8%) in the *H. pylori* negative group and 2 (7.7%) in the *H. pylori*
Fig 4.2.5. Pre and post supplementation levels of plasma alpha tocopherol in *H. pylori* negative and positive subjects.

Pre and post supplementation, change, and proportional change of plasma alpha tocopherol by quartiles of pre supplementation levels distribution in *H. pylori* negative and positive groups are presented in table 4.2.7 and illustrate in fig. 4.2.6. In the *H. pylori* negative group, proportional change decreased with increasing pre supplementation levels of alpha tocopherol (132.25±41.42, 108.40±37.45, 64.55±38.96, 26.57±35.59 for the 1\(^{st}\), 2\(^{nd}\), 3\(^{rd}\) and 4\(^{th}\) quartiles respectively; p for trend <0.05). A comparison between *H. pylori* negative and positive group showed that, in the 2\(^{nd}\) quartile, the change and proportional change in plasma alpha tocopherol in *H. pylori* negative were significantly higher compared to the *H. pylori* positive group (change: 20.00±7.82 vs. 6.96±13.05 respectively; proportional
change: 108.40±37.45 vs. 33.33±60.96 respectively). This finding was despite the significantly lower pre supplementation levels in the *H. pylori* negative compared to the positive group (18.39±1.86 vs. 21.17±1.49 respectively). (Table 4.2.7).
Table 4.2.7 Pre and post supplementation levels, change and proportional change of plasma alpha tocopherol by quartiles of pre supplementation levels distribution in *H. pylori* negative and positive subjects†.

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Pre supplementation (umol/l)</th>
<th>Post supplementation (umol/l)</th>
<th>p-value*</th>
<th>Change† (umol/l)</th>
<th>Proportional Change † (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (8)</td>
<td>14.35±0.95</td>
<td>33.22±5.62</td>
<td>&lt;0.001</td>
<td>18.87±5.62</td>
<td>132.25±41.42</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (7)</td>
<td>17.37±2.046</td>
<td>29.89±4.69</td>
<td>&lt;0.001</td>
<td>12.52±4.11</td>
<td>73.02±24.85</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.01</td>
<td>0.23</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (9)</td>
<td>18.39±1.86</td>
<td>38.38±8.67</td>
<td>&lt;0.001</td>
<td>20.00±7.82</td>
<td>108.40±37.45</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>21.17±1.49</td>
<td>28.13±13.06</td>
<td>0.95</td>
<td>6.96±13.05</td>
<td>33.33±60.96</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.01</td>
<td>0.25</td>
<td>0.06</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (9)</td>
<td>23.89±1.93</td>
<td>39.08±8.63</td>
<td>0.001</td>
<td>15.19±8.80</td>
<td>64.55±38.96</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (8)</td>
<td>25.40±2.04</td>
<td>37.77±27.66</td>
<td>0.02</td>
<td>12.37±11.21</td>
<td>48.49±43.97</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.14</td>
<td>0.69</td>
<td>0.58</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> –ve (8)</td>
<td>30.85±5.34</td>
<td>38.36±10.01</td>
<td>0.09</td>
<td>7.51±10.70</td>
<td>26.57±35.59</td>
</tr>
<tr>
<td><em>H. pylori</em> +ve (6)</td>
<td>31.81±2.53</td>
<td>58.85±11.76</td>
<td>0.01</td>
<td>27.04±25.62</td>
<td>81.36±71.71</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.58</td>
<td>0.08</td>
<td>0.13</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from paired t test comparing pre and post supplementation levels of plasma ascorbic acid (log transformed data).
** p-value derived from independent t test comparing *H. pylori* negative and positive groups (log transformed data).
Fig 4.2.6. Pre and post plasma alpha tocopherol concentration by quartiles of plasma alpha tocopherol at baseline in *H. pylori* negative and positive subjects

Analysis of adjusted plasma alpha tocopherol plasma (mg/g lipids) are presented in appendix Q. The results of this analysis were similar to the unadjusted analysis. They indicated a significant increase in plasma alpha tocopherol in *H. pylori* negative and positive groups following supplementation, and no significant differences in pre and post supplementation levels, change and proportional change between the two groups.
4.2.3.2 Plasma malondialdehyde (MDA) (umol/l)

Overall 121 out of 122 blood samples were analyzed for plasma MDA. One subject’s post supplementation blood sample for MDA was missing; therefore this subject was not included in the analysis. All values for proportional change in plasma MDA were within 3 SDs of its mean and therefore none was excluded (mean ± SD: -13.37 umol/l ± 35.43) (Fig 4.2.7).

Fig 4.2.7 Proportional change (%) in plasma MDA for all subjects.

Plasma MDA concentrations for all subjects were very low (<0.1 umol/l), thus the data will be represented with 3 decimal points for a better description. Post supplementation levels of plasma MDA were lower than pre supplementation levels in both groups but the difference reached statistical significance only in the *H. pylori* negative group (*H. pylori* negative: 0.103±0.006 vs. 0.015±0.014; p<0.05; *H. pylori* positive: 0.009±0.006 vs.
0.011±0.010; ns). Pre and post supplementation plasma MDA levels were not significantly different between *H. pylori* negative and positive groups (table 4.2.8).

Table 4.2.8 Pre and post supplementation plasma MDA in *H. pylori* negative and positive subjects†.

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> –ve (n=34)</th>
<th><em>H. pylori</em> +ve (n=26)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation</td>
<td>0.015±0.014</td>
<td>0.011±0.010</td>
<td>0.11</td>
</tr>
<tr>
<td>(umol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post supplementation</td>
<td>0.010±0.006</td>
<td>0.009±0.006</td>
<td>0.51</td>
</tr>
<tr>
<td>(umol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value**</td>
<td>0.002</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from independent t test for comparison of means between *H. pylori* negative and positive group (log transformed data).
** p-value derived from paired t test for comparison of pre and post supplementation plasma levels (log transformed data).

Two subjects had relatively high pre supplementation plasma MDA (0.78 and 0.53 umol/l) (Fig 4.2.8). The analysis without these subjects was carried out and the results did not change neither in direction nor in significance, therefore they were kept in the analysis. Fig 4.2.8 illustrates pre and post supplementation levels for each subjects in the *H. pylori* negative and positive group.
Fig 4.2.8. Pre and post supplementation levels of plasma MDA in *H. pylori* negative and positive subjects
4.2.3.2 Plasma thiols

All 122 plasma samples were analyzed for their thiols content. Two subjects had post supplementation plasma thiol levels below zero, as a result their pre and post supplementation data were not included in the analysis. Another two subjects had extreme values of proportional change (294.64% and -91.36%). These value were greater than the mean ± 3SDs (mean ± SD: 6.68 ± 54.0) and therefore were excluded (fig. 4.2.10).

Fig 4.2.10. Proportional change (%) in plasma thiols in all subjects

Post supplementation levels of plasma thiols were not significantly different than pre supplementation levels in *H. pylori* negative and positive groups (*H. pylori* negative: 512.48 ±176.67 vs. 501.72 ± 136.26 respectively *H. pylori* positive: 492.92 ± 155.06 vs. 521.85± 171.61 respectively). Comparison of *H. pylori* negative and positive groups showed that pre and post supplementation plasma thiols were comparable between the two groups (table 4.2.10),
Table 4.2.9. Pre and post supplementation plasma thiols (umol/l) in *H. pylori* negative and positive subjects†.

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> –ve (n=32)</th>
<th><em>H. pylori</em> +ve (n=26)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation (umol/l)</td>
<td>501.72 ± 136.26</td>
<td>521.85 ± 171.61</td>
<td>0.59</td>
</tr>
<tr>
<td>Post supplementation (umol/l)</td>
<td>512.48 ± 176.67</td>
<td>492.92 ± 155.06</td>
<td>0.38</td>
</tr>
<tr>
<td>p-value**</td>
<td>0.94</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from independent t test for comparison of means between *H. pylori* negative and positive group (log transformed data).
** p-value derived from paired t test for comparison of pre and post supplementation plasma levels (log transformed data).

Fig. 4.2.11 Pre and post supplementation levels of plasma thiols in *H. pylori* negative and positive subjects

H. pylori negative          H. pylori positive
Chapter 5: Discussion


5.1.1 Prevalence of *H. pylori* infection in Ontario

To our knowledge, this is the first study to estimate *H. pylori* prevalence in an adult Canadian population. Since most prevalence studies have not weighted their estimates, this discussion will be limited to the un-weighted data. We found an overall seroprevalence of 29.4%. Veldhuyzen et al. (1994) from Halifax reported a higher seroprevalence in a sample of 316 subjects (38%). In his sample, prevalence rates increased with age: 39.2%, 41.1%, 47.2% and 50% in the 5th, 6th, 7th and 8th decades respectively. The CADET-PE study group found a comparable prevalence of 30% among 1100 dyspeptic patients (Thomson et al., 2003). This is unexpected because *H. pylori* infection is generally more common in dyspeptic patients compared to the general population (Veldhuyzen et al., 1994; Kuipers et al., 1995; Laine L., 2002); a possible explanation may be the younger sample in the CADET-PE study where the mean age was 45.8 years compared to 64.7 years for our OFCCR controls. Another explanation may be the different sensitivity of the method of diagnosis. Serological testing was used in this study whereas endoscopy and the urea breath test were used by in CADET-PE. The latter two tests detect active infection, while serology does not differentiate between current and past infection. Therefore, the use of serology may lead to an overestimation of prevalence by including subjects who had been infected but were cured prior to testing. Other studies that investigated the prevalence of *H. pylori* in Canada were restricted to high risk groups; therefore their estimates were expected to be higher than our results. A study of the
Inuit communities in Wasagamack in the Canadian Arctic reported prevalences as high as 95% for adults and 56.4% among children aged 6 weeks to 12 years old (Sinha et al., 2002). Another study of two traditional Inuit communities in the central Canadian arctic found prevalence rates ranging between 32.1% for children <15 years old and 64.3% for adults between 60-74 yrs respectively (Mc Keown et al., 1999).

The prevalence estimate in this Canadian sample (29.4%) was comparable to values from other developed countries. In the United States, Everhart et al. (2000) reported a seroprevalence of 32.5% among 7465 adults, aged 20 to 59 years, participating in the Third National Health and Nutritional Examination Survey (1988-1991). In Australia, Robertson et al. (2003) reported a seroprevalence of 32% in a sample of blood donors whose mean age was 40 years. In England and Wales, 14% of all the population was estimated to be ever infected. However, subjects born before the 1950’s had a seroprevalence of 30% (Vyse et al., 2002), which is comparable to our estimate.

When weighted by sex and 5 year age groups to the distribution of the Ontario population (2003), the overall seroprevalence estimate dropped to 23.1%. The distribution of the OFCCR controls is skewed to the older age groups because of the age match by 5 or 10 years of age to colon cancer cases. Given the fact that H. pylori infection is more common in older individuals, it was expected that the non-weighted prevalence estimate be higher than that of the total general population.

5.1.2 Association of various factors with H. pylori infection

Age and the risk of H. pylori infection
Worldwide, two characteristic age-specific patterns of *H. pylori* seroprevalence have been described: in developing countries infection appears to occur early in life with chronic infection continuing into adulthood (Frenck et al., 2003), while in developed countries the prevalence among children is low, but rises throughout adult life (Lindkvist et al., 1996; Rothenbacher et al., 2003). In our study, seroprevalence rates followed the pattern of other developed countries with prevalence higher in older than younger people and highest with people of at least 70 years of age. Whether this pattern is a result of a constant infection rate over time or of a birth cohort effect, with decreasing rates in subsequent generations could not be determined given the cross sectional nature of this survey. Potential reasons for decreasing *H. pylori* prevalence with subsequent cohorts include improved hygiene and nutrition during childhood, smaller family size, larger time interval between children and increase consumption of antimicrobials (Tytgat, G., 1999). Various cohorts studying the pattern of *H. pylori* infection with age produced with conflicting results. The study by Veldhuyzen et al. (1994) from Halifax, Canada, speculated that the increasing prevalence of infection with age may be a result of a continuous risk of infection in adults rather than a cohort effect. After a 3 year follow up period during which at least 2 blood samples were obtained for each of 316 subjects, the authors showed that a 1% increase in risk of acquisition each year best explains the pattern of *H. pylori* infection in the Canadian population they have studied. On the other hand, Roosendaal et al. (1997) gave evidence for a continuous decrease of infection rates from childhood onward, based on cross sectional data. They determined the *H. pylori* infection status in 314 serum samples from Dutch children and young adolescents collected between 1978 and 1993. In children in the age group of 6-8
years, the prevalence of *H. pylori* declined from 19% to 9% between 1978 and 1993. In young adolescents, a decline of *H. pylori* infection from 23% to 11% was observed.

**Sex**

Our results indicated a significant effect of sex on prevalence. Females were at a lower risk of infection; even after adjustment for other risk factors. This finding is in accordance with many reports from the literature where males were found to have significantly higher infection rates than females (Perez-Perez et al., 1991; Graham et al., 1991; Repogle et al., 1995; Fawcett et al., 1996; Staat et al., 1996; Lin et al., 1998). This may explain, in part, the male predominance of *H. pylori* adult diseases, like duodenal ulcer and gastric cancer. A recent meta-analysis of population-based prevalence surveys aiming to assess the association between *H. pylori* and sex showed that in 18 adult populations the male sex was significantly associated with the infection (OR: 1.16, CI: 1.1-1.2) while in pediatric populations (10) there was no difference in prevalence between girls and boys (Martel et al., 2006). The effect of sex on *H. pylori* infection may be related to either a difference in the incidence rate of the infection or in the rate of infection loss. The results of the previously mentioned meta-analysis do not support a differential rate of incidence of infection as there was no effect of sex in paediatric studies. A more plausible explanation is the greater loss of infection in females, which may be due to a stronger protective immune response to *H. pylori* in females or a greater female exposure to curative antibiotic treatment (Klein, 2004). Several studies illustrate that immunological differences exist between the sexes that may underlie increased infection in males. Females typically have higher immune responses than males (Zuk et al., 1996). Field and laboratory studies link sex differences in immune function with circulating steroid hormones (Klein S., 2000). In general, estrogen stimulates immune
response, while testosterone is immunosuppressive (Nalbandian et al., 2005). Increased susceptibility to infection is thought to be one of the leading causes of increased death rates among men as compared with women (Owens et al., 2002).

The other explanation for the lower infection rates of *H. pylori* in females is the possibility that adult females are more likely to have infection eradicated with antimicrobials used for other illnesses (Luknarova et al., 1992). In British Columbia, Canada, women consumed 17% more antibiotics than males (Patrick et al., 2004). A cohort study in New Zealand showed that in the year before age 21, self-reported antibiotic use was more frequent among females (Thomson et al., 2002). Other evidence for a differential loss of the infection emerges from resistance studies where bacterial infection in females were shown to be more resistant to clarithromycin and metronidazole, suggesting past exposure to these antibiotics (Meyer et al., 2002).

**Ethnicity, place of birth and age at immigration.**

Ethnic differences in *H. pylori* infection rates are well documented. Our results showed that non-white males had higher prevalence rates than white males. These findings echoed the results of other studies of risk factors for *H. pylori* infection. In the United States, Everhart et al., (2000) showed that Mexican American and non-Hispanic black males had higher prevalence of the infection when compared to non- Hispanic white males (63.4%, 58.3% and 27.5% respectively). In Malaysia, a study of 188 males and females found that, compared to Malays, Indians had the highest odds of *H. pylori* infection (OR: 11.6, CI: 7.09-18.8) followed by Chinese (OR: 4.8, CI: 3.04-7.56) (Rajendra et al., 2007). Our results showed that the differences of prevalence between whites and non whites were attenuated
after adjustment for other factors, which suggest that the effects of ethnicity and place of birth are surrogates for other risk factors, such as education and socioeconomic status.

Furthermore, we found that prevalence rates were higher among immigrants. In the study by Everhart et al. (2000) it was shown that being born outside the United States increased odds of infection to 2.5. In addition, Latin Americans who were born in the United States or Canada had substantially lower infection prevalence than those born outside these countries (Staat et al., 1996). In a study of *H. pylori* infection in different generations of Hispanics in the San Francisco Bay area, Tsai et al., (2005) showed that the prevalence of the infection in immigrants and first and second generations US born Hispanics were 31.4%, 9.1% and 3.1%, respectively. Few studies investigated the effect of the age of immigration on prevalence of infection status. Our results indicate higher prevalence estimates among subjects who immigrated at least 20 years of age compared to those who immigrated at younger ages. This effect remained even after adjustment for other risk factors. These findings agree with the results of the study by Akin et al., (2004) in Turkey, which showed that immigration between the ages of 20-29 and >30 increased the risk of infection compared to immigration at ages younger than 9 years old (OR: 1.9; CI: 1.64-2.18 and OR: 1.73; CI: 1.48-2.01, respectively). This effect of the age of immigration suggest that exposure to the infection among immigrants is highest in the first 20 years of life, which is characteristic feature of *H. pylori* infection in developing countries.

When stratified by sex, the effect of these demographic variables was shown to be significant only for males. It seems that females are less affected by the geographical demographics and more dependent on other factors, such as age and number of siblings. The reason why females’ rates of infection were not affected by the place of birth, ethnicity and
the age of immigration is not clear. To our knowledge, no studies have looked at the relation between *H. pylori* infection and these variables for each sex separately.

**Number of siblings**

Increased number of siblings is among the well-established risk factors for *H. pylori* infection (Everhart et al., 2000; Akin et al., 2004; Koch et al., 2005; Santos et al., 2005). In our data, the number of siblings was positively associated with infection, even after adjustment for other risk factors. Males and females from families with greater than 2 and siblings had higher odds of being infected than smaller families. Since the number of siblings and socioeconomic status are highly correlated, it has been suggested that the former effect is confounded by socioeconomic variables such as education and income (Rowland M., 2000). In our data, however, the number of siblings remained a significant risk factor even after adjustment for income and education. It is not known whether the observed effect of the number of siblings is due to sharing a common exposure source or transmission among individuals. In an effort to elucidate the intra-familial transmission of *H. pylori* infection, Weyermann et al. (2006) suggested that the mother may be the main source of infection and that the increased infection prevalence in families with a large number of siblings is largely due to children sharing a common exposure: the mother. The odds ratios for *H. pylori* infection of the child were 12.9, 95% CI: 3.2-52.5 if the mother was infected and 1.4, 95%CI: 0.4-4.6 if the father was infected.

**Education and Income**

Among the OFCCR controls, the income and education variable were moderately correlated (r= 0.3). Both of these variables, as indicated in our results, are inversely associated with the incidence of *H. pylori* infection, although the association is not always
significant. These findings are in accordance with the risk factors studies for the infection, most of which confirm a negative association with these variables (Santos et al., 2005; Everhart et al., 2000).

The highest number of missing answers was for the income question: 482 out of 1306 (37%). This observation is not uncommon in socio-demographic questionnaires. What is of interest, however, is the lower prevalence among subjects with missing answers compared to those who completed the income question. Analysis of demographic characteristics revealed that the missings are significantly younger, more educated and have fewer siblings than subjects who answered the income questions. This difference in the demographic distribution between the two groups may explain in part the lower prevalence of *H. pylori* infection in the missing group.

<table>
<thead>
<tr>
<th>Table 5.1.1 Comparison of demographic characteristics between subjects who answered the income question and those who did not.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects who answered the income question</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Education</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Number of siblings</td>
</tr>
<tr>
<td>&lt;2 siblings</td>
</tr>
<tr>
<td>2-4 siblings</td>
</tr>
<tr>
<td>&gt;4 siblings</td>
</tr>
</tbody>
</table>

*P-Value derived from t-test  
**P-Value derived from Chi-square test

**Alcohol**

Among lifestyle factors, we found that alcohol intake was associated with a decrease in *H. pylori* prevalence. All drinkers had significantly lower odds of infection compared to
subjects who never drank. These findings are in accordance with several reports in the literature that assessed the relationship between alcohol consumption and *H. pylori* infection. A pooled analysis of three studies from southern Germany, comprising 1410 adults aged 15 to 69, showed that prevalence of current *H. pylori* infection was lower among subjects who consumed alcohol (34.9%) than among non-drinkers (38.0%), regardless of the type of alcoholic beverages consumed (Brenner et al., 2001). In 2002, the report of the Bristol Helicobacter Project found a negative association between consumption of wine and beer and *H. pylori* infection prevalence in 10,537 participants. After adjustment for age, sex, ethnic status, childhood and adult social class, smoking and coffee consumption, subjects drinking 3-6 servings of wine/week had an 11% lower risk of infection compared with those who took no wine. These findings were similar for beer consumption (Murray et al., 2002). Given the fact that acquisition of *H. pylori* infection occurs in childhood, the inverse association between alcohol consumption and *H. pylori* infection would more likely reflect suppression or elimination of the infection by alcohol consumption rather than reduced rates of acquisition of the infection. Antimicrobial effects of alcohol beverages have long been noted (Ingram et al., 1984; Weisse et al., 1995) and there is increasing evidence from recent in vitro studies that alcoholic beverages may effectively inhibit the growth of *H. pylori* (Marimon et al., 1998). Furthermore, consumption of alcohol increases gastric acid secretion and speeds gastric emptying, which could aid eradication of *H. pylori* (Bujanda, L., 2000)

**Smoking**

Our results echoed other reports from the literature, which showed no relationship between *H. pylori* infection and smoking (Constanza et al., 2004; Santos et al., 2005; Everhart et al., 2000; Murray et al., 2002). However Akin et al., (2004) in Turkey found a
positive association between quitting smoking and the prevalence of the infection (OR: 2.15; CI: 1.55-2.99). Further investigations of the positive association between \textit{H. pylori} infection and quitting smoking revealed that 89.7\% of the quitters did so because of a health problem (Tezcan et al., 2003). Therefore the observed positive effect of quitting on the prevalence of the infection may be an artifact of a confounding effect of other health problems, which may be related to the \textit{H. pylori} infection.

\textit{Regular use of antacids, aspirin and acetaminophen}

Antacids are ingested to relieve heartburn, the major symptom of peptic ulcer. Our results showed that \textit{H. pylori} infection was more prevalent among males who regularly used antacids, even after adjustment for age, age at immigration and number of siblings. This finding was expected given the fact that \textit{H. pylori} infection is associated with peptic ulcer (Laine L., 2002). The reason for the lack of association between intake of antacids and \textit{H. pylori} prevalence in females is unclear. No previous studies reported on the prevalence of antacids in relation to \textit{H. pylori} infection.

Several studies linked the incidence and severity of headaches to \textit{H. pylori} infection (Tunca et al., 2004; Gasbarrini et al., 1998). A review of the literature by Pellicano et al. (2007) showed that in intervention studies, at 6 and 12 months, eradication of the bacterium was associated to disappearance with symptoms in 23\% and 28\% respectively, and with a significant decrease of intensity, frequency and duration of acute attacks in the remaining patients. This association was not found in observational studies. Since aspirin and acetaminophen are two commonly used medications for headache relief, it was of interest to investigate possible association between the regular use of these drugs and the infection. Our
results suggested no association between *H. pylori* infection and regular aspirin or acetaminophen use.

**BMI, fruits, vegetables and meat intakes**

Recently, there has been an increasing interest in the consequences of *H. pylori* infection on BMI. It is postulated that infection may decrease gastric ghrelin and increase gastric leptin levels, which may, in turn, decrease the BMI (Liew et al., 2006). Our results indicated no relationship between BMI and infection. These findings are similar to other reports from the literature. From the United States, Ioannou et al., (2005) screened 6724 adult participants of the Third National Health and Nutrition Examination Survey for *H. pylori* infection. They found no association between *H. pylori* seropositivity and BMI in this population. An earlier study in Japan examined the relationship between *H. pylori* seropositivity and BMI in 932 employees of an industrial corporation. The results showed no association (Azuma et al., 2002). These findings may reflect the fact that substantial alterations in the ghrelin and leptin levels are only observed when infection induces gastric atrophy (Masaoka et al., 2005)

Our results indicated no association between intakes of fruits, vegetables and meats and prevalence of *H. pylori* infection. Few reports have addressed these associations and their findings are contradictory. In a sample of 5861 Mexican subjects aged 11-21 years old, intakes of fruits, vegetables, meats and milk products were associated with the incidence of *H. pylori* infection (Constanza et al., 2004). Olafsson et al., (2003) showed that after eradication of *H. pylori* in 183 peptic ulcer and gastritis patients aged between 28 and 82 years, consumption of fruits increased from 4.0 to 4.3 servings/week, while consumption of vegetables and meats did not change.
**Prevalence of diabetes**

It has been proposed that severe gastric inflammation or ulcer disease can alter gastric motility and influence glycemic control in patients with type 2 diabetes mellitus (Boehme et al., 2007). However, data on the prevalence of *H. pylori* infection in diabetics are scanty and contradictory. The prevalence of *H. pylori* infection has been variously reported as high (Perdichizzi et al., 1996; Gasbarrini et al., 1998), low (Malecki et al., 1996) and normal (Gillum RF, 2004; Anastasios et al., 2002; Woodward et al., 2000; Hia et al., 2001) in diabetic patients. Our results are in line with the observations that indicated no relationship between *H. pylori* infection and diabetes. The variability of prevalence rates among diabetic patients compared to controls may be related to the epidemiological distribution of *H. pylori* and failure to control for factors that affect both the prevalence of *H. pylori* and diabetes such as age and sex. Furthermore, different inclusion criteria for patient enrollment in various studies might have contributed to the discrepancies in their results; these criteria are related to the age at onset and the duration of the disease.

**5.1.3 Study strengths and limitations**

To date, there have been few population-based studies that have examined the prevalence of *H. pylori* infection in Canada. Important strengths of this study were its large sample size (the largest prevalence study to date in Canada) and the inclusion of a large number of demographic and lifestyle variables. The target population, to which this study aims to generalize its results, is the Ontario population ages 50 to 80 years old.

A test and treat strategy for *H. pylori* infection that results in decreased incidence of gastritis, gastric atrophy, intestinal metaplasia and gastric cancer would probably target adults
between 45 and 50 years old (Sullivan et al., 2004). Studies of the efficacy of *H. pylori* eradication in preventing gastric cancer have suggested the presence of “point of no return” before which treatment of the infection may result in the regression of already existing gastric lesions. This point of no return may occur at any age after the infection and before the occurrence of the cancer (De Vries et al., 2007). Given that the median age at diagnosis of gastric cancer is around 70 years old (Crew et al., 2006), studying adults between 50 and 80 years of age is important in evaluating the efficacy of a test and treat strategy in the Canadian context.

### 5.1.3.1 Selection bias

The OFCCR controls were recruited from across Ontario. According to Statistics Canada (2001), fifteen percent of the general population in Ontario lives in rural areas. By definition, a rural population for 1981 to 2001 refers to persons living outside centres with a population of 1,000 and outside areas with 400 persons per square kilometer (Statistics Canada, 2001). The distribution of the OFCCR controls was assessed by postal codes of home address. As specified by Canadapost, a postal code with “0” in the second character of the Forward Sortation Area (FSA) designated a rural area. We found that 13.2% of the OFCCR controls lived in rural areas. Therefore the distributions of the OFCCR controls and the general population in Ontario were not considerably different and do not affect the generalizability of the prevalence estimates of *H. pylori* infection obtained in this study.

A potential limitation of this study is the fact that the participants were selected from lists that do not have a 100% coverage rate of the target population.
The listing of households in the first phase (1999-2000) of recruitment was obtained from Infodirect, a subsidiary of Bell Canada. Households without telephones, or with unlisted telephone numbers, were excluded from the sample frame while households with multiple listed telephone numbers were represented more than once. Given that the coverage rate of this list exceeds 90%, the extent to which these differences affect the results of the prevalence estimate should not be significant.

In the second phase of recruitment (2002-2003), the Year 2000 Ministry of Finance Assessment File was used to select controls. This file included residents of Ontario who pay residential taxes (whether owned or rented residential property). Migration in or out of Ontario may precipitate a bias in the coverage of this file, however given that it is updated every four months, the effect of migration is therefore insignificant. Another source of bias when using this file is the fact that homeless people are not represented.

The response rate was 20.6% (15.21% and 34.4 % for phase I and II respectively). Ineligibility to participate and language problems accounted for the majority of non-responders (47.8%), followed by refusal to participate (21.4%), and inability to reach (10.2%). Since there was no information on characteristics the non-responders, it was not feasible to assess the possible effect of this response rate on *H. pylori* infection prevalence estimates.

The generalizability of the prevalence estimate of *H. pylori* infection obtained in this study is tied to the comparability of the OFCCR controls sample to the general population in Ontario. Table 5.1.2 represents selected demographic characteristics of the OFCCR controls sample and of the general population in Ontario as per the census data in 2001, in addition to
an estimate of the overall prevalence should the OFCCR controls have the same distribution as the general population in Ontario for the specific demographic characteristics.
Table 5.1.2 Selected demographic characteristics of the OFCCR controls and the general population of Ontario and the expected overall seroprevalence of *H. pylori* infection†.

<table>
<thead>
<tr>
<th></th>
<th>OFCCR controls sample</th>
<th>General population in Ontario</th>
<th>Overall seroprevalence expected†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>733 (56.1 %)</td>
<td>5,577,060 (48.88%)</td>
<td>29.0%</td>
</tr>
<tr>
<td>Females</td>
<td>573 (43.9%)</td>
<td>5,832,990 (51.12%)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1210 (92.79%)</td>
<td>9,132,500 (80.92%)</td>
<td>30.25%</td>
</tr>
<tr>
<td>Non white</td>
<td>94 (7.21%)</td>
<td>2,153,045 (19.08%)</td>
<td></td>
</tr>
<tr>
<td><strong>Place of birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Canada</td>
<td>858 (65.70%)</td>
<td>8,164,855 (72.35%)</td>
<td>28.51%</td>
</tr>
<tr>
<td>Outside Canada</td>
<td>448 (34.30%)</td>
<td>3,120,695 (27.65%)</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>506 (39.28%)</td>
<td>5,003,320 (55.29%)</td>
<td>30.26%</td>
</tr>
<tr>
<td>Medium</td>
<td>447 (34.7%)</td>
<td>2,269,190 (25.08%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>335 (26.01%)</td>
<td>1,775,530 (19.62%)</td>
<td></td>
</tr>
</tbody>
</table>

†The overall seroprevalence expected is an estimate of the seroprevalence of *H. pylori* infection should the OFCCR controls have a similar distribution to the general population in Ontario according to given demographic characteristic.

The prevalence estimates of *H. pylori* infection should the OFCCR controls have similar distributions to the general population of Ontario range between 29.0 and 30.26%. Given that the overall prevalence estimate obtained in this study is 29.4%, it seems that the differences in the demographic distribution between our study sample (OFCCR controls) and the reference population (general population in Ontario) have little effect on the generalizability of our results.

5.1.3.2 Differences among blood givers and non-givers

Another important consideration is the differences between blood givers and non-givers among the OFCCR controls. The table below presents a comparison between the blood
givers and non-givers among the OFCCR controls according to selected demographic variables. This comparison is based on 1306 blood givers and 628 blood non-givers, whose data was available at the time this study took place.

Table 5.1.3. Demographic characteristics of blood givers and non-givers in the OFCCR control population.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Level</th>
<th>Blood givers</th>
<th>Blood non-givers</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>64.06±8.66 (n=1306)</td>
<td>57.3±10.51 (n=628)</td>
<td>p&lt;0.01*</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>733 (56.1%)</td>
<td>302 (48%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>573 (43.9%)</td>
<td>326 (51.9%)</td>
<td>p&lt; 0.01**</td>
</tr>
<tr>
<td>Education</td>
<td>Low</td>
<td>506 (39.28%)</td>
<td>269 (43%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>447 (34.7%)</td>
<td>206(33%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>335 (26.01%)</td>
<td>150 (24%)</td>
<td>p= 0.11**</td>
</tr>
</tbody>
</table>

*P-Value derived from t-test
**P-Value derived from Chi-square test

The blood givers were older than blood non-givers, and the proportion of males was greater among blood-givers than non-givers.

A sensitivity analysis by age group and sex was conducted to estimate the range for the overall prevalence of the infection for various age groups should all the OFCCR controls gave blood (Table 5.1.4). The non-givers range of prevalence was chosen to be comparable to reports from the United States and England of same sex and age intervals (Everhart et al., 2000; Vyse et al., 2002).
Table 5.1.4. Prevalence estimate in the OFCCR blood givers and the range of the overall prevalence in the OFCCR controls should the non-givers participated.

<table>
<thead>
<tr>
<th>Age</th>
<th>Prevalence among blood givers</th>
<th>Range of prevalence of blood non-givers*</th>
<th>Range of prevalence (blood givers and non-givers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60 years</td>
<td>23.9%</td>
<td>20%-30%</td>
<td>17%-31%</td>
</tr>
<tr>
<td>60-70 years</td>
<td>28.4%</td>
<td>25%-35%</td>
<td>21%-33%</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>37.7%</td>
<td>35%-45%</td>
<td>33%-38%</td>
</tr>
</tbody>
</table>

*These ranges are taken from various studies in the literature of *H. pylori* prevalence of similar sex and age intervals.

5.1.3.3 Other limitations

Another possible limitation on the interpretation of findings arises because there was no collection of data on whether subjects had received specific anti-*H. pylori* therapy. Using serology to identify *H. pylori* infection, would lead to overestimation of the prevalence of current infection. However, since the subjects were recruited from the general population and were not necessarily symptomatic, the proportion of subjects who had previously received a successful eradication therapy is likely to have been low. Given the large sample size studied, this limitation has limited effects on the results of this study.

It would have been valuable for the interpretation of the results to obtain further information about the controls, such incidence of peptic ulcer or frequency and intensity of headache. However since the Personal History Questionnaire was pre-set and data was collected prior to the start of this study, obtaining such information was not possible and the interpretation was limited to the available demographic, lifestyle and medical data already included in the questionnaire.
The cross sectional nature of this study makes it impossible to determine whether age is a risk factor for the infection, or whether the observed increase in infection prevalence with age is a cohort effect. In addition, the cross sectional design does not allow conclusions about causality of observed associations between *H. pylori* and lifestyle factors, such as in the case of the negative association with alcohol consumption.

### 5.1.4 Summary

The overall seroprevalence of *H. pylori* infection in a sample of adults from Ontario aged 50 to 80 years is 29.4%, with males having a higher prevalence (33% for males and 28.4% for females). The weighted overall seroprevalence was 23.1%. Weighting the seroprevalence yielded lower estimates given the fact that *H. pylori* infection is more common in older individuals and that the OFCCR controls populations age distribution is skewed to the right (towards older age). The findings of this study are in harmony with the results of previous research on the association between *H. pylori* infection with age, ethnicity, place of birth, number of siblings and alcohol consumption. In addition, a positive association was found between the infection prevalence and age at immigration. Our results confirmed a higher seroprevalence among males, and suggest a sex difference in the associations between *H. pylori* infection and various demographic and lifestyle factors.
5.2 Project II: Effect of \textit{H. pylori} infection on the bioavailability of vitamins E and C

The aim of this study was to assess the effect of \textit{H. pylori} infection on the bioavailability of vitamins E and C. A simple test for bioavailability of vitamins E and C is by assessing the increase in plasma levels of these vitamins following their oral intake (Lodge, J., 2005). The results from this study showed that \textit{H. pylori} negative and positive subjects had similar levels of ascorbic acid, alpha tocopherol, MDA and thiols at baseline. After supplementation with 500 mg vitamin C and 400 IU alpha tocopherol for 28 days, plasma levels of the vitamins increased significantly in both groups. Contrary to our hypothesis, \textit{H. pylori} negative and positive groups were not significantly different in either the change or proportional change of plasma ascorbic acid and alpha tocopherol. In addition, both groups had similar plasma MDA and thiols concentrations at baseline as well as after supplementation.

5.2.1 Demographic characteristics of \textit{H. pylori} negative and positive groups

Our results indicated that \textit{H. pylori} positive subjects were slightly older and had a higher percentage of married individuals compared to \textit{H. pylori} negative subjects. These differences did not reach statistical significance and do not have an important impact on the bioavailability of vitamins E and C. The two groups were similar with regard to BMI. Sex distribution was significantly different between the groups, with higher percentage of males in the \textit{H. pylori} positive group. A negative subject was recruited whenever a positive subject was recruited, provided that the negative subject had been screened within one week time
interval. This method of selecting the negative subjects was carried out in order to avoid seasonal variation among participants, including variation in food consumption (especially fruits and vegetables), and various stress triggers (e.g., exams, weather). This method made it infeasible to match negative and positive subjects on age and sex. The decision not to give priority to age and sex matching was taken for the following reasons. First, age was already restricted to a range between 18 and 45 years in the inclusion criteria of participation, and there is no evidence of effect of age on vitamin C bioavailability in this age range. Second, sex was not expected to be a confounder. In order to confound the results, sex would need to be related both to infection and to plasma ascorbic acid. While a recent meta-analysis of population-based prevalence surveys aiming to assess the association between *H. pylori* and sex showed that in adult populations the male sex was significantly associated with the infection (OR: 1.16, CI: 1.1-1.2) (Martel et al., 2006), the inverse association between the male sex and plasma ascorbic acid is still controversial (Preston et al., 2006; Wrieden et al., 2000). In our study, there was no association between sex and any of the plasma measures of ascorbic acid, alpha tocopherol, MDA and thiols, as indicated by a p-value>0.05 for the independent t test of means with sex as the grouping variable and the plasma measures as independent variables (appendix R).

### 5.2.2 Dietary intakes of *H. pylori* negative and positive groups

In humans, diet is a major determinant factor of plasma ascorbic acid and alpha tocopherol. As plasma ascorbic acid and alpha tocopherol concentrations are correlated with the intakes of these vitamins (vitamin C: r=0.43; vitamin E: r=0.12) (Willett W., 1998), it was important to control for the potential confounding effect of dietary intakes of the vitamin E
and C in studying the association of supplementation and the plasma levels of these vitamins. Intake of vitamin C in *H. pylori* negative and positive groups was close to RDA values of the vitamin (90 mg for adult men and 75 mg for adult women) both at week 0 and week 4 of the study. In addition it was similar to estimates of median dietary intakes of vitamin C for adults in the United States and Canada (102 mg/day and 72 mg/day respectively) (IOM, 2000). For both the *H. pylori* negative and positive groups, vitamin E intake, at week 0 and week 4 of the study, was lower than the RDA, 15 mg/day for both men and women. This observation may be due to three sources of measurement error that are important with regard to vitamin E intake: (1) energy intake may be underreported (Mertz et al., 1991), and fat intake (which serves as a major carrier for vitamin E) is likely to be more underreported than energy intake (Briefel et al., 1997); (2) the amount of fats and oils added during food preparation (and absorbed into the cooked product) is difficult to assess, yet contributes substantially to vitamin E intake; (3) the particular fats or oils consumed are uncertain, particularly when food labels do not provide the specific fat or oil in the product and necessitate a reliance on default selections (and thus assumptions about the relative content of alpha tocopherol).

The analysis of the 7-day food records indicated that energy adjusted intake of vitamin E was similar between *H. pylori* negative and positive groups at baseline and during week 4 of the study. For vitamin C, there was a 20% difference between the two groups at week 0, yet this difference did not reach statistical significance due to small sample size. This finding of no association between dietary intake and *H. pylori* infection agreed with the results of a cross sectional study of 5861 young adult Mexicans (Constanza et al., 2004).

The only significant difference in dietary intakes between the two groups was the lower energy adjusted fiber consumption among the *H. pylori* positive group. The literature
on the relationship of fiber intake and *H. pylori* infection is very limited. Among the symptoms of *H. pylori* infection is bloating. Adverse effects of fiber use may include abdominal discomfort and bloating (Zuckerman MJ., 2006). Therefore, a possible explanation for the difference in fiber consumption is the fact that *H. pylori* positive subjects may have a lower tolerance for fiber containing food items because these foods aggravate an existing discomfort.

Furthermore, our results showed no change in vitamin E and C consumption during supplementation (week 4) as compared to consumption in week 0 in both *H. pylori* negative and positive groups. This finding is supported by another study of 439 middle aged men that found no effect of five year supplementation of vitamin C on the dietary consumption of the vitamin during this period (Kim et al., 2003).

### 5.2.2 Effect of supplementation on plasma measures

*H. pylori* infection increases the production of ROS and RNS in the gastric medium which may lead to the consumption of dietary antioxidants. The effect of *H. pylori* infection on the bioavailability of two main dietary antioxidants, vitamins E and C, was assessed by comparing the change in the plasma levels of ascorbic acid and alpha tocopherol after supplementation with these vitamins between *H. pylori* negative and positive groups. It remains important to establish that plasma levels of ascorbic acid and alpha tocopherol reflect changes in gastric levels of these vitamins. Few studies examined this relationship; Sanderson et al., (1997) showed that plasma and gastric concentrations of alpha tocopherol are correlated; $r = 0.69$, $p < 0.0003$. Henry et al. (2005) demonstrated that consumption of PPIs, which are known to decrease gastric levels of ascorbic acid, also decreased significantly
plasma levels of ascorbic acid (12%) in *H. pylori* negative and positive subjects (Henry et al., 2005).

### 5.2.2.1 Ascorbic acid

In total, 3 out of 120 samples (2.5%) had implausible values for ascorbic acid content in plasma. This is not uncommon in laboratory assays carried out manually. Mishandling of the sample before or during the analysis of the blood sample may have caused oxidation and ultimately loss of ascorbic acid. Although every effort was made to ensure accurate copying of the values from the spectrophotometer, another possible source of error is reading and recording the values of plasma ascorbic acid.

*Pre vs. post supplementation plasma ascorbic acid in *H. pylori* negative and positive groups*

Pre supplementation levels of plasma ascorbic acid in *H. pylori* negative and positive subjects were within the normal range (22-85 umol/l). In both groups, supplementation with 500 mg vitamin C produced a significant increase in plasma levels of ascorbic acid (44% and 67% in *H. pylori* negative and positive groups respectively). These findings agree with the results of other studies in the literature that investigated the effect of oral supplementation of vitamin C on its plasma levels. A review of supplementation trials with doses of vitamin C ranging from 250 mg/day to 1000 mg/day for periods varying between one week and two months produced up to a 4 fold increase in plasma levels of the vitamin (IOM, 2000). In addition, a recent study by Bader et al. (2007) showed that supplementation with 500 mg
vitamin C and 182 mg alpha tocopherol for 4 weeks increased plasma levels of ascorbic acid by about 30-40% in 19 non-smoker healthy men volunteers aged 19-39 years.

**Plasma ascorbic acid in H. pylori negative vs. H. pylori positive groups**

Contrary to our hypothesis that *H. pylori* infection would reduce the bioavailability of the vitamin, our results indicated that 1) *H. pylori* negative and *H. pylori* positive groups had similar plasma levels of ascorbic acid at baseline and 2) post supplementation plasma levels, change and proportional change were slightly higher, though not significantly, in the infected group.

The relationship between *H. pylori* infection and plasma ascorbic acid has been the subject of many observational and experimental studies possibly due to the fact that ascorbic acid is a major antioxidant in the gastric medium where *H. pylori* exists. However, the literature around this relationship is inconsistent. Our results agree with studies that showed no effect of the infection on the plasma levels of the vitamin. In accordance with our findings, Phull et al. (1998) demonstrated that, after adjustment for sex and smoking status, there was no relationship between *H. pylori* infection and plasma levels of ascorbic acid, alpha tocopherol, malondiadehyde in 43 patients undergoing routine endoscopy for investigation of dyspepsia. Similarly, an earlier report in 1997 from the EUROGAST study by Webb et al. found no difference in plasma vitamin C between *H. pylori* infected and non infected subjects among 1,404 subjects from 9 European centers (Webb et al., 1997).

Other studies found a negative association between *H. pylori* infection and plasma levels of vitamin C. In a sample of 1168 subjects aged 25-74 years selected from general practitioner’s lists in Glasgow, Woodward et al. (2001) found the mean plasma vitamin C concentration of *H. pylori* positive subjects to be 65% that of those who were classified as
negative. This difference increased to 80% after correction for confounding factors, such as age, sex, social class, smoking and vitamin C intake. Similar results were obtained from the Third National Health and Nutrition Examination Survey in the United States, where the authors found that higher serum ascorbic acid were associated with a decreased seroprevalence of *H. pylori* infection among adult whites (Simon et al., 2003).

This inconsistency of the literature on the relationship between *H. pylori* infection and plasma ascorbic acid might be explained by stage of infection and the strain of *H. pylori*.

Park et al., (2003) looked at the correlation between *H. pylori* infection and vitamin C levels in whole blood, plasma and gastric juice in Korean children and found no significant difference in plasma ascorbic acid between those who were *H. pylori* negative (n=248) and *H. pylori* positive (n= 204) (72.11 ± 24.41 vs. 77.79±24.98 umol/l respectively). However, when ranked according to histologic density of the infection, the severity of *H. pylori* infection was inversely associated with plasma vitamin C levels (r = - 0.82; p<0.001).

Another factor that may influence the effect of *H. pylori* infection on plasma vitamin C levels is the strain of the bacteria, particularly the strain that expresses the cag A virulence factor. The Third National Health and Nutrition Examination Survey examined the effect of cag A strain on serum ascorbic acid levels in 6746 adults. After adjustment for seroprevalence of *H. pylori* infection, a 0.50 mg/dl (28.38 umol/l) increase in serum ascorbic acid levels among whites was associated with a decrease in seroprevalence of the pathogenic cag A positive strain of *H. pylori* (OR: 0.31, 95% CI: 0.12-0.79; p<0.05). Although the direction of this relation was similar in non whites, the confidence interval was wide, and the association was not statistically significant (OR: 0.26; 95% CI: 0.0-5.14; p=0.36) (Simon et al., 2003).
In our study, there was no information on the stage on the infection, its severity or the strain of the bacterium. It may have been the case that, because our subjects were relatively young, they had less severe infection, which might explain the lack of effect of the infection on plasma levels of ascorbic acid.

Our results indicated, contrary to our hypothesis, a slightly, though not significant, higher absolute change and proportional change in plasma ascorbic acid in *H. pylori* positive group. This greater effect observed in the *H. pylori* positive group may be due to chance, as indicated by the not-statistically significant p and log transformed p values (>0.05), or it may be that higher magnitude of change in that group did not reach statistical significance due to limited sample size. If *H. pylori* is not associated with plasma ascorbic acid, the results would be consistent with the previous finding of no significant difference in plasma ascorbic acid between *H. pylori* negative and positive groups at baseline. However, if *H. pylori* positive status was associated with increased change in plasma ascorbic acid, an increased absorption of DHAA may explain this greater change in plasma ascorbic acid seen among *H. pylori* positive subjects. Depending on the location of the infection of *H. pylori* in the stomach, the pH in the gastric medium may be affected (Calam et al., 2001). Several specialized cells in the gastric muscosa contribute to the control of acid secretion including G cells, enterochromaffin-like cells, parietal cells and somatostatin cells. The predominantly antral infection with *H. pylori* leads to acid hypersecretion by suppressing somatostatin cells and increasing gastrin release from the G cells in the gastric antrum (Harris et al., 1996). While infection in the corpus of the stomach mainly affect parietal cells leading to a low acid secretion and consequently a higher pH (Saponin et al., 1996). Ascorbic acid is unstable in the presence of increased pH, and is converted to the less active form of DHAA. The
mechanisms of DHAA uptake by luminal membranes of human jejunum has pharmacological characteristics that clearly differ from those of ascorbic acid uptake. Sodium-independent carriers take up DHAA by facilitated diffusion, and these are distinct from the sodium dependent transporters of ascorbic acid. In our study, absorption of DHAA by *H. pylori* infected subjects may have been important given the limited capacity of enterocytes sodium dependent transporters of ascorbic acid. Infection with *H. pylori* may therefore have increased the conversion of ascorbic acid to DHAA in the gastric medium and consequently its intestinal absorption and reduction by the enterocytes. This effect of *H. pylori* infection would only be observed when vitamin C was supplemented because it is in this situation that the transporters of ascorbic acid become saturated and the absorption of DHAA becomes significant. It is, therefore, of interest that future research on the effect of *H. pylori* on vitamin C absorption assesses the uptake of DHAA and ascorbic acid separately.

5.2.2.2 Plasma alpha tocopherol

*Pre vs. post supplementation plasma alpha tocopherol in H. pylori negative and positive groups*

At baseline, *H. pylori* negative and positive groups had plasma levels of alpha tocopherol within the normal range, between 12 and 46 umol/l. Supplementation with 400 IU alpha tocopherol produced a significant increase in the plasma levels of alpha tocopherol in both groups (83.15% and 58.79% in *H. pylori* negative and positive groups respectively). These results are similar to the findings of previous supplementation studies of similar doses of alpha tocopherol. In healthy volunteers of both sexes aged 26-64 years, supplementation with 400 IU of alpha tocopherol for 28 days increased plasma levels of alpha tocopherol by
79.4% of baseline concentration (Dimitrov et al., 1996). Another study of 38 healthy subjects, increases in plasma alpha tocopherol (as a proportion of baseline plasma levels) of 5%, 40.2%, 47.8% and 51% were observed with doses of 22.5, 150, 300, and 600 IU of alpha tocopherol supplementation respectively over 21 days (Eichhorn et al., 2004).

*Plasma alpha tocopherol in H. pylori negative vs. H. pylori positive groups*

Our results showed that, at baseline and after supplementation, *H. pylori* negative and positive groups had similar levels of plasma alpha tocopherol. Change and proportional change in plasma alpha tocopherol were similar in both groups. Our findings support the results of two studies which showed no relationship between *H. pylori* infection and plasma alpha tocopherol. Sanderson et al. (1997) compared alpha tocopherol levels in plasma between 21 *H. pylori* negative and 23 positive subjects. The results showed little difference in plasma alpha tocopherol between the infected and non-infected groups. Similar findings were observed by Phull et al. (1996) who found no effect of *H. pylori* infection on plasma alpha tocopherol among 30 patients undergoing routine gastroscopy for investigation of dyspepsia.

In contrast, Zhang et al., 2000 found a decrease in alpha tocopherol in the gastric mucosa of *H. pylori* infected subjects compared to normal controls, with a stepwise decrease in patients with atrophy and intestinal metaplasia. Nair et al., 2000 found that, regardless of the infection status, patients with gastritis and ulcer had significantly lower serum alpha tocopherol compared to controls. Thus it seems that gastric lesions, rather than *H. pylori* infection, may be associated with plasma alpha tocopherol concentrations.
5.2.2.3 Plasma malondialdehyde and thiols

*Pre and post supplementation plasma malondialdehyde and thiols in* *H. pylori* negative and positive groups.

The effect of *H. pylori* infection on oxidative stress was assessed by measuring plasma MDA and plasma thiols in *H. pylori* negative and positive subjects. Our results indicated lack of oxidative stress in both groups as reflected by very low plasma MDA and within normal range plasma thiols. In addition, there existed no significant differences in plasma MDA or thiols between *H. pylori* negative and positive subjects before or after supplementation with 500 mg vitamin C and 400 IU of alpha tocopherol.

In our study, plasma MDA levels for *H. pylori* negative and positive were low compared to other reports from the literature. The type of anticoagulant used in plasma collection seems to affect plasma MDA levels (Suttnar et al., 2001). In our study, EDTA was used as the anticoagulant. EDTA plasma MDA measurements produce lower values than heparin, citrate, and serum samples (Nielson et al., 1997; Carbonneau et al., 1991; Wong et al., 1987; Knight et al., 1987). This is probably due to the chelation of iron by EDTA that limits in vitro peroxidation. Iron is necessary in the superoxide-driven Fenton reaction and in the iron catalyzed Haber-Weiss reaction that produces hydroxyl radicals leading to increased lipid peroxidation and increased levels of MDA (Aruma et al., 1991). Studies using EDTA plasma will then detect lower values of MDA than studies using heparin, citrate, or vials without antiocoagulants when sampling blood.

Nielsen et al., (1997) determined reference intervals for plasma from a randomly selected group of 480 persons from the Danish Central Personal registry, selected to include
an equal number of men and women, ages 20-79 year, and equal participation from individuals living in urban and rural municipalities. Blood samples were collected using EDTA tubes. The authors found the 90% confidence interval for plasma MDA for subjects aged 20 to 40 years to be 0.30-0.36 umol/l. The results showed that smokers had higher plasma MDA than non smokers (0.66 umol/l vs. 0.60 umol/l; p=0.05), in addition plasma MDA was significantly correlated with weekly alcohol consumption (r=0.153; p=0.03).

Our results revealed plasma MDA levels lower than the study described above, possibly because none of our subjects was a smoker and alcohol drinking was limited to three drinks per day as per the exclusion criteria. The low plasma MDA we observed suggests that our subjects did not have significant oxidative stress. This finding is confirmed by the plasma thiols results.

Plasma thiol levels were within normal range (400-600 umol/l) before and after supplementation in both *H. pylori* negative and positive groups. Plasma thiols are reduced in diseases which produce higher oxidative stress. Fleshner et al., (2000) found significantly lower plasma levels of thiols among 24 cases of prostate cancer compared to 18 controls (p<0.05). Yilmaz et al., (2003) compared 43 male bladder cancer patients to 28 healthy controls and found that plasma thiols were significantly lower among cases (399.56 ± 81.58 vs. 451.45 ± 53.04; p<0.05). Further, patients with invasive bladder cancer had significantly lower plasma thiols than patients presenting with non invasive cancer (341.86 ± 91.80 vs. 416.05 ± 71.93; p <0.05).

The low levels of MDA and normal thiols levels in plasma we observed suggest that the healthy young participants in this study were not under oxidative stress. Although our results showed that supplementation with 500 mg vitamin C and 400 IU significantly
decreased plasma MDA in the *H. pylori* negative group, this decrease is not clinically significant. Therefore our supplementation regime appears not to have reduced oxidative stress in our subjects, probably because of the low level of such stress in these subjects at baseline. Our findings are similar to the observations in several studies that supplementation with antioxidants does not have an effect on oxidative stress in healthy individuals (Carty et al., 2000; Hininger et al., 2001; Meagher et al., 2001).

*Plasma MDA and thiols in *H. pylori* negative vs. *H. pylori* positive groups*

Our results did not show an effect of *H. pylori* infection on oxidative stress measures as reflected by similar pre and post supplementation levels of MDA and thiols in the *H. pylori* negative and positive groups. This finding echoed the results of Phull et al., 1998, who found that, after controlling for sex and smoking status, there was no significant difference in plasma MDA between *H. pylori* negative and positive subjects in 43 dyspeptic patients. In contrast, two studies found that infection with *H. pylori* increases oxidative stress measures (Drake et al., 1998; Khanzode et al., 2003). Drake et al. (1998) found that after 4 weeks of successful eradication of *H. pylori* infection in 161 dyspeptic patients, gastric levels of MDA decreased significantly. Khanzode et al. (2003) compared 37 *H. pylori* gastritis and 30 gastric cancer patients to 40 healthy volunteers and found that plasma MDA was significantly increased in *H. pylori* gastritis and gastric cancer patients compared to healthy volunteers (mean ± SEM: 6.06 ±0.27; 3.12 ±0.07; 1.74±0.10 for gastric cancer, *H. pylori* gastritis and controls respectively).
As was previously described for the association between *H. pylori* infection and plasma antioxidants, the observed association between *H. pylori* infection and oxidative stress may be explained by the severity of infection and the strain of the bacteria. A study by Farinati et al., (1998) showed that the severity of gastritis, assessed from gastric biopsies, is positively associated with oxidative stress as gastric MDA (nmol/g tissue) was increased with the severity of the disease (37.9±15.3; 39.8±21.3; 58.6±39.5 for disease severity of +, ++, and +++ respectively).

The relationship between *H. pylori* infection and oxidative stress is also affected by the strain of the bacteria and specifically the presence of a functional cag A pathogenicity island. Several studies have concluded that infection with cag A positive *H. pylori* strains is associated with higher levels of mononuclear and neutrophilic infiltrates, more severe atrophy, and intestinal metaplasia and alterations in the gastric epithelial cell cycle, and apoptosis (Nogueria et al., 2001; Peek et al., 1999; Moss et al., 2001; Kidd et al., 1999). Farinati et al. (2003) showed that cag A positive strains were associated with higher concentration (p<0.01) of 8-hydroxydeoxyguanosine (8OHdG) in the gastric muscosa (a marker of mutagenic/carcinogenic oxidative DNA adducts).

### 5.2.3 Summary

Our results failed to show an effect of *H. pylori* infection on the change and proportional change in plasma ascorbic acid and alpha tocopherol upon supplementation with 500 mg vitamin C and 400 IU vitamin E. This finding was confirmed by the lack of association between *H. pylori* infection and oxidative stress as measured by plasma MDA and plasma thiols. The discrepancies between these findings and the literature may be
explained by the fact that the effect of *H. pylori* infection on oxidative stress and consequently on vitamins E and C arises from the severity of the infection and the strain of the bacterium. In this study, infected subjects were young and had no overt symptoms of gastritis; therefore it could be assumed that their infection was in its early stages and not yet severe. We did not obtain information on the strain of the bacterium in *H. pylori* infected subjects, thus were unable to control for the effect of the cagA positive strains.

5.3 Conclusions and recommendations

*H. pylori* infection has been recognized as having a prominent causative role in the etiology of gastric cancer. There is, however, no conclusive evidence that *H. pylori* eradication prevents gastric cancer. Supportive data could, theoretically, be obtained from prospective trials of eradication, but in practice, such trials do not exist and may be impossible to conduct for reasons of duration and ethics. Consequently decisions will most likely be based on accumulation of evidence from observational epidemiological and biological studies. A knowledge gap analysis in the field of *H. pylori* research revealed two main areas that are still obscure: prevalence of the infection and its pathogenecity. The purpose of this thesis was to shed light on these areas in *H. pylori* infection research in Canada, including assessment of *H. pylori* infection prevalence in Ontario and exploration of the potential effect of the bacterium on the bioavailability of the main dietary antioxidants, vitamins E and C.

Assessment of the prevalence of the infection in Ontario was critical in determining its magnitude and its public health burden. Ontario is a province located in the east central part of Canada that has the largest population and is the second largest province (after
Quebec) in total area (Statistics Canada, 2007). We found an overall prevalence of *H. pylori* infection in a sample of Ontario adults aged 50 to 80 years to be 29.4%. Males had higher prevalence than females (33.0% vs. 28.4%). Subjects who were older and belonged to larger families were at a higher risk of the infection. Factors associated with the incidence of *H. pylori* infection were different among males and females. For males, being born outside Canada and immigrating at an age older than 20 years old and taking antacids regularly increased the risk of being infected; whereas for females, alcohol intake seemed to be negatively associated with the incidence of the infection. This study is the first step in the demonstration project proposed by Sullivan et al. (2004), in which screening would be offered to adults in Ontario within specified upper and lower age limits the infection. Eradication therapy will be offered to *H. pylori* infected subjects. The results of this project will allow the development of a measure of the number of people that would require *H. pylori* treatment to prevent one case of gastric cancer (number needed to screen) and ultimately determine the costs and benefits of *H. pylori* eradication (incidence of stomach cancer, gastro-esophageal reflux disease and peptic ulcer).

Given its complications (e.g., atrophic gastritis and gastric cancer), *H. pylori* infection endangers public health. The results of this study helped define a high risk population of older immigrants from large families. Educational programs could be planned and implemented on topics such as personal hygiene, nutritional hygiene, transmission routes of *H. pylori*, and relevant preventive measures. Further research might focus on the effectiveness of screening and treating immigrants upon landing in Canada.

Whether the observed increase of infection rates with age is a result of a higher rate of acquisition or a birth cohort effect is still to be determined. Future studies looking at infection
rates over time might answer the question. In addition, research on both physiological and behavioral levels is warranted. Women tend to have lower infection rates and lower gastric cancer incidence, but to date we have no explanation for this observation.

The assessment of the prevalence of *H. pylori* infection, although giving important information on the magnitude of the infection in the population, has to be coupled with research in infection pathogenesis in order to better assess the weight of its burden. Recently, oxidative stress has been suggested to be a major pathological pathway for *H. pylori*. The clinical outcome of *H. pylori* infection varies according to the interrelation of bacterial, host and environmental factors. Therefore, studying the pathological pathways of *H. pylori* in light of its interaction with dietary factors is a promising field that may explain the various clinical outcomes of this infection. Vitamins E and C are the major dietary antioxidants that are postulated to have a protective role against gastric cancer. The results of our second study showed that *H. pylori* infection does not reduce the bioavailability of vitamins E and C, neither does it increase oxidative stress in asymptomatic subjects. These findings do not back any recommendation to increase the recommended intakes of the vitamins in case of *H. pylori* infection.

We have suggested that the severity of the infection may influence the effect of *H. pylori* infection on the bioavailability of vitamins E and C. Future research is warranted on the effect of *H. pylori* on bioavailability of these vitamins in gastric lesions with varying severity. In addition controlling for the strain of the infection may be important in assessing its effect on bioavailability of vitamins E and C.

Our results showed that, in asymptomatic “healthy” subjects, *H. pylori* did not increase oxidative stress, at least as measured by plasma MDA and thiols. This finding may
imply that this infection is not harmful in its early stages until a factor or combination of factors trigger the inflammation cascade which ultimately might lead to gastric cancer. In this case future research should focus on uncovering these factors, whether host, bacterial or a combination of both. Another implication of our finding is that asymptomatic *H. pylori* infection, although it does not increase oxidative stress as indicated by indices of oxidation measured in this study, it may predisposes to gastric cancer by mechanisms other than overproduction of reactive oxygen and nitrogen species, in which case research on pathological mechanisms other than oxidative stress is needed.
References:


Azuma T, Suto H, et al. (2002). Eradication of *Helicobacter pylori* infection induces an increase in body mass index. *Aliment Pharmacol Ther*; 16 (suppl 2): 240-244


Manolakis A, Andreas N, et al. (2007). A Review of the Postulated Mechanisms Concerning the Association of *Helicobacter pylori* with Ischemic Heart Disease


Appendices
Appendix A: Ethics approval letter from University of Toronto Ethics Board for study 1 “H. pylori infection in Ontario: prevalence and risk factors”.

[Image of the ethics approval letter]

[Text of the ethics approval letter]

UNIVERSITY OF TORONTO
Office of the Vice-President, Research and Associate Provost
Ethics Review Office

PROTOCOL REFERENCE #13231

January 20, 2005

Dr. N. Kreiger
Senior Epidemiologist
Cancer Care Ontario
620 University Ave., 15th Floor
Toronto, ON M5G 2L7

Ms. F. Naja
30 Charles St. W., Apt. 1009
Toronto, ON M4Y 1R5

Dear Dr. Kreiger & Ms. Naja:

Re: Your research protocol entitled, “The Prevalence of Helicobacter Pylori Infection in Ontario” by Dr. N. Kreiger (supervisor), Dr. M. Cothet, Dr. T. Sullivan (co-investigators), Ms. F. Naja (PhD candidate)

ETHICS APPROVAL

Original Approval Date: January 20, 2005
Expiry Date: January 19, 2006

We are writing to advise you that a member of the Health Sciences II Research Ethics Board has granted approval to the above-named research study, for a period of one year, under the Board’s expedited review process. Ongoing projects must be renewed prior to the expiry date.

We acknowledge receipt of the Mount Sinai Hospital Research Ethics Board approval letter for this study with an expiry date of December 14, 2005

During the course of the research, any significant deviations from the approved protocol (that is, any deviation which would lead to an increase in risk or a decrease in benefit to participants) and/or any unanticipated developments within the research should be brought to the attention of the Ethics Review Unit.

Best wishes for the successful completion of your project.

Yours sincerely,

Marianna Richardson
Ethics Review Coordinator

[Signatures and contact information]
Appendix B: Ethics approval letter from Mount Sinai Hospital Ethics Board for study 1 “H. pylori infection in Ontario: prevalence and risk factors”.

December 14, 2004
Ms. Nancy Kreiger
Cancer Care Ontario
620 University Ave.
12th floor
Toronto, Ontario
MSG 2L7

Dear Ms. Kreiger:

RE: MSH REB # 04-0267-E
Prevalence in Helicobacter Pylori Infection in Ontario

The above named proposal has received expedited review by the Mount Sinai Hospital Research Ethics Board. The proposal is approved for the next 12 months. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB should be notified of the termination of this study and a final report provided.

If, during the course of the research, there are any changes in the approved protocol or any new information that must be considered with respect to the study, these should be brought to the immediate attention of the Board. As the Principal Investigator, you are responsible for the ethical conduct of this study.

Sincerely,

Ronald Heslegrave, Ph.D.
Chair, Mount Sinai Hospital Research Ethics Board

14 December, 2004
Date of Initial REB Approval

14 December, 2005
Expiry Date of REB Approval

The Mount Sinai Hospital REB operates in compliance with the Tri-Council Policy Statement and CIHR/GCP Guidance and the REB requirements as defined in Canada's Food and Drug Act, Division 5

A University of Toronto affiliated patient care, teaching and research centre
Appendix C: Personal History Questionnaire of the Ontario Familial Colon Cancer Registry
This questionnaire is about factors that may relate to a person’s risk of developing cancer. Although it is important to have complete data for scientific reasons and we encourage you to answer all questions, if you come to a question that you do not want to answer, please write “prefer not to answer” beside it and then continue to answer the remaining questions.

Should you wish to talk to someone about this questionnaire, you may call (416) 217-1310 or 1-866-225-2728.
Please write in your answers where space is provided, or place tick marks in circles.

What date are you filling out this questionnaire? ____/____/____  

day  month  year

Identifying Information

1. Are you male or female?  
○ male  
○ female

2. What is your age?  ____ ____ years  
○ don’t know

3. What is your date of birth?  
   day  ____ ____  
   month  ____ ____  
   year  ____ ____ ____ ____  
○ don’t know day  
○ don’t know month  
○ don’t know year

4. Are you a twin or triplet?  
○ yes, a twin  
○ yes, other multiple (triplet, quadruplet, etc.): _______________  
○ no  
○ don’t know

   If yes, please read the following statement and answer the question.

   Non-identical twins are no more alike than ordinary brothers and sisters. Genetically identical twins, on the other hand, look so much alike (that is, they have a strong resemblance to each other in height, colouring, features of the face, etc.) that people often mistake one for the other, especially during their childhood.

   Do you have a genetically identical twin or triplet?  
○ yes  
○ no  
○ don’t know

5. What is your marital status?  
○ currently married or living as married  
○ separated  
○ divorced  
○ widowed  
○ single or never married  
○ don’t know
Bowel Screening and Health

6. Have you ever had a test for blood in your stool, called a smear test or a hemoccult? This test is frequently done as part of a routine physical examination, or it can be done at home.

- yes
- no → Please go to #7
- don’t know → Please go to #7

6a. When did you first have this test?

- age when first tested _____
- or
- year of first test ______ ______
- don’t know

6b. What were the reasons for your first test? Please tick all that apply.

- to investigate a new problem
- family history of colorectal cancer
- routine/yearly examination or check-up
- follow-up of a previous problem
- other: ____________________ please specify
- don’t know

6c. How many times have you had a hemoccult test?

- _____ number of hemoccult tests
- don’t know

6d. If you have had a hemoccult test more than once, when did you last have this test?

- age when last tested _____
- or
- year of last test ______ ______
- don’t know

7. Have you ever had a sigmoidoscopy? Sigmoidoscopy involves looking inside the lower bowel and rectum with a lighted instrument. This examination is usually done in a doctor’s office without anesthesia.

- yes
- no → Please go to #8
- don’t know → Please go to #8

7a. When did you first have this test?

- age when first tested _____
- or
- year of first test ______ ______
- don’t know

7b. What were the reasons for your first sigmoidoscopy? Please tick all that apply.

- to investigate a new problem
- family history of colorectal cancer
- routine/yearly examination or check-up
- follow-up of a previous problem
- other: ____________________ please specify
- don’t know

7c. How many times have you had a sigmoidoscopy?

- _____ number of sigmoidoscopies
- don’t know

7d. If you have had a sigmoidoscopy more than once, when did you last have this test?

- age when last tested _____
- or
- year of last test ______ ______
- don’t know
8. Have you ever had a colonoscopy?
Colonoscopy is an examination of the entire large bowel using a long flexible instrument. This examination is usually done under sedation.
- yes
- no ➔ Please go to #9
- don’t know ➔ Please go to #9

8a. When did you first have this test?
- age when first tested _____ _____
- or
- year of first test _____ _____ _____
- don’t know

8b. What were the reasons for your first colonoscopy? Please tick all that apply.
- to investigate a new problem
- family history of colorectal cancer
- routine/yearly examination or check-up
- follow-up of a previous problem
- other: ______________________
  please specify
- don’t know

8c. How many times have you had a colonoscopy?
- _____ number of colonoscopies
- don’t know

8d. If you have had a colonoscopy more than once, when did you last have this test?
- age when last tested _____ _____
- or
- year of last test _____ _____ _____
- don’t know

9. Has a doctor ever told you that you had polyps in your large bowel or colon or rectum? Polyps are growths in the lining of the colon which vary in size from a tiny dot to several inches.
- yes
- no ➔ Please go to #10
- don’t know ➔ Please go to #10

9a. When did your doctor first tell you that you had polyps?
- age at first diagnosis _____ _____
- or
- year of first diagnosis _____ _____ _____
- don’t know

9b. Have you been told more than once that you had polyps?
- yes
- no
- don’t know

9c. When did your doctor last tell you that you had polyps?
- age at last diagnosis _____ _____
- or
- year of last diagnosis _____ _____ _____
- don’t know

9d. Do you know what kind of polyps they were? Please include all the separate times you were told you had polyps. Please tick all that apply.
- benign
- adenomatous (pre-cancerous)
- hyperplastic
- other: ______________________
  please specify
- don’t know
9e. Did you have the polyps removed (by a procedure called a polypectomy)? (This can be done during a sigmoidoscopy or colonoscopy.)
- yes
- no \(\rightarrow\) Please go to \#10
- don’t know \(\rightarrow\) Please go to \#10

9f. When did you first have polyps removed?
- age when first polypectomy \_\_\_\_\_\_\_
- or
- year of first polypectomy \_\_\_\_\_\_\_\_
- don’t know

9g. Have you had polyps removed more than once?
- yes
- no
- don’t know

9h. If you have had polyps removed more than once, when did you last have polyps removed?
- age at last polypectomy \_\_\_\_\_\_\_
- or
- year of last polypectomy \_\_\_\_\_\_\_\_
- don’t know

10. Has a doctor ever told you that you had familial adenomatous polyposis, known also as FAP? This is a condition, sometimes occurring in families, in which numerous polyps line the inside of the large bowel or colon.
- yes
- no \(\rightarrow\) Please go to \#11
- don’t know \(\rightarrow\) Please go to \#11

10a. When did your doctor first tell you that you had FAP?
- age at diagnosis \_\_\_\_\_\_\_
- or
- year of diagnosis \_\_\_\_\_\_\_\_\_
- don’t know

11. Has a doctor ever told you that you had Crohn’s disease? This is where you have an inflammation that extends into the deeper layers of the intestinal wall. It may also affect other parts of the digestive tract, including the mouth, esophagus, stomach, and small intestine.
- yes
- no \(\rightarrow\) Please go to \#12
- don’t know \(\rightarrow\) Please go to \#12

11a. When did your doctor first tell you that you had Crohn’s disease?
- age at diagnosis \_\_\_\_\_\_\_
- or
- year of diagnosis \_\_\_\_\_\_\_\_\_\_
- don’t know

12. Has a doctor ever told you that you had ulcerative colitis? This is an inflammation and ulceration of the lining of the bowel (colon) and rectum. It is not a stomach ulcer.
- yes
- no \(\rightarrow\) Please go to \#13
- don’t know \(\rightarrow\) Please go to \#13

12a. When did your doctor first tell you that you had ulcerative colitis?
- age at diagnosis \_\_\_\_\_\_\_
- or
- year of diagnosis \_\_\_\_\_\_\_\_\_\_
- don’t know

13. Has a doctor ever told you that you had irritable bowel syndrome? This is a disorder of the bowels leading to cramping, gassiness, bloating and alternating diarrhea and constipation. It is sometimes called IBS, or spastic colon.
- yes
- no \(\rightarrow\) Please go to \#14
- don’t know \(\rightarrow\) Please go to \#14
13a. When did your doctor first tell you that you had irritable bowel syndrome?
   age at diagnosis ____ ____
   or
   year of diagnosis ____ ____
   ○ don’t know

14. Has a doctor ever told you that you had diverticular disease? This may also be called diverticulosis or diverticulitis. It’s a condition in which the bowel may become infected, and can lead to pain and chronic problems with bowel habits.
   ○ yes
   ○ no  → Please go to #15
   ○ don’t know  → Please go to #15

14a. When did your doctor first tell you that you had diverticular disease?
   age at diagnosis ____ ____
   or
   year of diagnosis ____ ____
   ○ don’t know

15. Have you ever had any of your large bowel or colon removed?
   ○ yes
   ○ no  → Please go to #15
   ○ don’t know  → Please go to #15

Was it completely removed, or was only part of it removed?
   ○ completely removed
   ○ partly removed
   ○ don’t know

15a. When did you first have any of your bowel or colon removed?
   age at first operation ____ ____
   or
   year of first operation ____ ____
   ○ don’t know

15b. Have you had more than one surgery to remove your bowel or colon?
   ○ yes
   ○ no  → Please go to #16
   ○ don’t know  → Please go to #16

15c. When did you last have all or part of your bowel or colon removed?
   age at last operation ____ ____
   or
   year of last operation ____ ____
   ○ don’t know

16. Have you had your gallbladder removed?
   ○ yes
   ○ no  → Please go to #17
   ○ don’t know  → Please go to #17

16a. When did you have your gallbladder removed?
   age at operation ____ ____
   or
   year of operation ____ ____
   ○ don’t know

17. Has a doctor ever told you that you had diabetes, also known as diabetes mellitus? Please do not include diabetes which you had only during pregnancy.
   ○ yes
   ○ no  → Please go to #18
   ○ don’t know  → Please go to #18

17a. When did your doctor first tell you that you had diabetes?
   age at diagnosis ____ ____
   or
   year of diagnosis ____ ____
   ○ don’t know
17b. Did you ever take medication to control your diabetes?
   - yes
   - no → Please go to #18
   - don’t know → Please go to #18

17c. What type of medication did you use, pills or insulin injections?
   - pills
   - insulin injections
   - both
   - don’t know → Please go to #18

17d. How often did you usually take it?
   Please choose the most appropriate category.

<table>
<thead>
<tr>
<th>Pills</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>times per day or</td>
<td></td>
</tr>
<tr>
<td>times per week or</td>
<td></td>
</tr>
<tr>
<td>times per month or</td>
<td></td>
</tr>
<tr>
<td>times per year</td>
<td></td>
</tr>
<tr>
<td>don’t know</td>
<td></td>
</tr>
</tbody>
</table>

17e. About two years ago, were you taking it?

<table>
<thead>
<tr>
<th>Pills</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td></td>
</tr>
<tr>
<td>don’t know</td>
<td></td>
</tr>
</tbody>
</table>

17f. How long, in total, have you taken this medication?

<table>
<thead>
<tr>
<th>Pills</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of months or</td>
<td></td>
</tr>
<tr>
<td>number of years</td>
<td></td>
</tr>
<tr>
<td>don’t know</td>
<td></td>
</tr>
</tbody>
</table>

18a. When did your doctor first tell you that you had high cholesterol?
   - age at diagnosis ______ ______
   - year of diagnosis ______ ______
   - don’t know

18b. Did you ever take medication to control your high cholesterol?
   - yes
   - no → Please go to #19
   - don’t know → Please go to #19

18c. How often did you usually take it?
   Please choose the most appropriate category.

<table>
<thead>
<tr>
<th>Pills</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>______ times per day or</td>
<td></td>
</tr>
<tr>
<td>______ times per week or</td>
<td></td>
</tr>
<tr>
<td>______ times per month or</td>
<td></td>
</tr>
<tr>
<td>______ times per year</td>
<td></td>
</tr>
<tr>
<td>don’t know</td>
<td></td>
</tr>
</tbody>
</table>

18d. About two years ago, were you taking it?
   - yes
   - no
   - don’t know

18e. How long, in total, have you taken this medication?

<table>
<thead>
<tr>
<th>Pills</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>______ number of months or</td>
<td></td>
</tr>
<tr>
<td>______ number of years</td>
<td></td>
</tr>
<tr>
<td>don’t know</td>
<td></td>
</tr>
</tbody>
</table>
19. Has a doctor ever told you that you had high levels of fat (other than cholesterol) in your blood, also called high triglycerides? If your doctor told you it was borderline, please tick no.
   ○ yes
   ○ no ➔ Please go to #20
   ○ don’t know ➔ Please go to #20

19a. When did your doctor first tell you that you had high triglycerides?
   age at diagnosis _____ _____
   or
   year of diagnosis _____ _____ _____
   ○ don’t know

19b. Did you ever take medication to control the high levels of fat in your blood?
   ○ yes
   ○ no ➔ Please go to #20
   ○ don’t know ➔ Please go to #20

19c. How often did you usually take it?
   Please choose the most appropriate category.
   _____ _____ times per day or
   _____ _____ times per week or
   _____ _____ times per month or
   _____ _____ times per year
   ○ don’t know

19d. About two years ago, were you taking it?
   ○ yes
   ○ no
   ○ don’t know

19e. How long, in total, have you taken this medication?
   _____ _____ number of months or
   _____ _____ number of years
   ○ don’t know

20. Has a doctor ever told you that you had any type of cancer?
   ○ yes
   ○ no ➔ Please go to #24
   ○ don’t know ➔ Please go to #24

20a. What type of cancer was it?
   ________________________cancer

20b. When did your doctor first tell you that you had this type of cancer?
   age at diagnosis _____ _____
   or
   year of diagnosis _____ _____ _____
   ○ don’t know

20c. Were you treated with radiation therapy (radiotherapy) for this cancer?
   ○ yes
   ○ no
   ○ don’t know

21. Has a doctor ever told you that you had any other cancer?
   ○ yes
   ○ no ➔ Please go to #24
   ○ don’t know ➔ Please go to #24

21a. What type of cancer was it?
   ________________________cancer

21b. When did your doctor first tell you that you had this type of cancer?
   age at diagnosis _____ _____
   or
   year of diagnosis _____ _____ _____
   ○ don’t know

21c. Were you treated with radiation therapy (radiotherapy) for this cancer?
   ○ yes
   ○ no
   ○ don’t know
22. Has a doctor ever told you that you had any other **cancer**?
   - yes
   - no → Please go to #24
   - don’t know → Please go to #24

22a. What type of cancer was it?
   ____________________cancer

22b. When did your doctor **first** tell you that you had this type of cancer?
   age at diagnosis _____ _____
   or
   year of diagnosis _____ _____ _____
   - yes
   - no
   - don’t know

22c. Were you treated with radiation therapy (radiotherapy) for this cancer?
   - yes
   - no
   - don’t know

23. Has a doctor ever told you that you had any other **cancer**?
   - yes
   - no → Please go to #24
   - don’t know → Please go to #24

23a. What type of cancer was it?
   ____________________cancer

23b. When did your doctor **first** tell you that you had this type of cancer?
   age at diagnosis _____ _____
   or
   year of diagnosis _____ _____ _____
   - yes
   - no
   - don’t know

23c. Were you treated with radiation therapy (radiotherapy) for this cancer?
   - yes
   - no
   - don’t know

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**Medications**

**Have you ever taken any of the following medications regularly (at least twice a week for more than a month)?**

- aspirin (such as Anacin, Bufferin, Bayer, Excedrin, Ecotrin)
  - yes
  - no → Please go to #25
  - don’t know → Please go to #25

24a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   _____ _____ times per day or
   _____ _____ times per week
   - yes
   - no
   - don’t know

24b. **About two years** ago, were you taking it regularly?
   - yes
   - no
   - don’t know

24c. How long, in total, have you taken this medication regularly? **If you started and stopped and then started again, please count only the time you were taking this medication.**
   _____ _____ number of months or
   _____ _____ number of years
   - yes
   - no
   - don’t know
25. Acetaminophen (such as Tylenol, Anacin-3, Panadol)?
   - yes
   - no → Please go to #26
   - don’t know → Please go to #26

25a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   - ____ ____ times per day
   - ____ ____ times per week
   - don’t know

25b. About two years ago, were you taking it regularly?
   - yes
   - no
   - don’t know

25c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   - ____ ____ number of months
   - ____ ____ number of years
   - don’t know

26. Ibuprofen-based medications (such as Advil, Motrin, Naprosyn, Medipren, Indocid, NSAIDS (NSAIDS are non-steroidal anti-inflammatory drugs))
   - yes
   - no → Please go to #27
   - don’t know → Please go to #27

26a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   - ____ ____ times per day
   - ____ ____ times per week
   - don’t know

26b. About two years ago, were you taking it regularly?
   - yes
   - no
   - don’t know

26c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   - ____ ____ number of months
   - ____ ____ number of years
   - don’t know
27. **Bulk-forming laxatives (such as Metamucil, Citrucel, FiberCon, Serutan, psyllium)?**
   - yes
   - no  →  Please go to #28
   - don’t know  →  Please go to #28

27a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   *Please choose one of the following.*
   - _____ _____ times per day or
   - _____ _____ times per week
   - don’t know

27b. **About two years** ago, were you taking it regularly?
   - yes
   - no
   - don’t know

27c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   - _____ _____ number of months or
   - _____ _____ number of years
   - don’t know

28. **Other laxatives (such as Ex-Lax, Correctol, Dulcolax, Senokot, Colace, castor oil, cod liver oil, mineral oil)?**
   - yes
   - no  →  Please go to #29
   - don’t know  →  Please go to #29

28a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   *Please choose one of the following.*
   - _____ _____ times per day or
   - _____ _____ times per week
   - don’t know

28b. **About two years** ago, were you taking it regularly?
   - yes
   - no
   - don’t know

28c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   - _____ _____ number of months or
   - _____ _____ number of years
   - don’t know
29. Multivitamin supplements (such as One-A-Day, Theragram, Centrum, Unicap)?
   (not individual vitamins)
   ○ yes
   ○ no → Please go to #30
   ○ don’t know → Please go to #30

29a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   ___ ___ times per day or
   ___ ___ times per week
   ○ don’t know

29b. About two years ago, were you taking it regularly?
   ○ yes
   ○ no
   ○ don’t know

29c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   ___ ___ number of months or
   ___ ___ number of years
   ○ don’t know

30. Folic acid or folate pill or tablets
   ○ yes
   ○ no → Please go to #31
   ○ don’t know → Please go to #31

30a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   ___ ___ times per day or
   ___ ___ times per week
   ○ don’t know

30b. About two years ago, were you taking it regularly?
   ○ yes
   ○ no
   ○ don’t know

30c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   ___ ___ number of months or
   ___ ___ number of years
   ○ don’t know

31. Calcium pills or tablets
   ○ yes
   ○ no → Please go to #32
   ○ don’t know → Please go to #32

31a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   ___ ___ times per day or
   ___ ___ times per week
   ○ don’t know

31b. About two years ago, were you taking it regularly?
   ○ yes
   ○ no
   ○ don’t know

31c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   ___ ___ number of months or
   ___ ___ number of years
   ○ don’t know

32. Calcium-based antacids (such as Tums, Rolaids, Extra-strength Rolaids, Alka-Mints, Chooz Antacid gum)
   ○ yes
   ○ no → If female. please go to #33
          If male. please go to #44
   ○ don’t know → If female. please go to #33
          If male. please go to #44

32a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
   Please choose one of the following.
   ___ ___ times per day or
   ___ ___ times per week
   ○ don’t know

32b. About two years ago, were you taking it regularly?
   ○ yes
   ○ no
   ○ don’t know

32c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.
   ___ ___ number of months or
   ___ ___ number of years
   ○ don’t know

Men: please go to #44 on page 17
Women: please continue with #33 on page 13
33. How old were you when you had your first menstrual period
   ____ ____ years of age
   ○ don’t know
   ○ never had a menstrual period

34. Have you ever been pregnant?
   ○ yes
   ○ no  
      Please go to #35
   ○ don’t know  
      Please go to #35

   How many times have you been pregnant?
   Please include miscarriages, stillbirths, tubal pregnancies and abortions.
   ____ ____ number of pregnancies
   ○ don’t know

34a. How many times were you pregnant with more than one baby (twins, triplets or more)? If you are pregnant now, please do not include your current pregnancy.
   ○ never
   ____ ____ number of pregnancies with more than one baby
   ○ don’t know

34b. How many of your pregnancies lasted 6 months or longer? (Pregnancy usually lasts 9 months. Six months is about the earliest a baby could survive.) If you are pregnant now, please do not include your current pregnancy.
   ○ all of them
   ____ ____ number of pregnancies lasting 6 months or longer
   ○ don’t know

34c. How many of your pregnancies resulted in live births?
   ○ all of them
   ____ ____ number of pregnancies with live-born children
   ○ don’t know

34d. How old were you at the first live birth?
   age at first birth ____ ____ or
   year of first birth ____ ____ ____ ____
   ○ don’t know

34e. How old were you at the last live birth?
   age at last birth ____ ____ or
   year of last birth ____ ____ ____ ____
   ○ don’t know

35. Have you ever used birth control pills or other hormonal contraceptives (implants or injections) for at least one year?
   ○ yes
   ○ no  
      Please go to #36
   ○ don’t know  
      Please go to #36

   How old were you when you first used any of these hormonal contraceptives?
   age at first use ____ ____ or
   year of first use ____ ____ ____ ____
   ○ don’t know

35a. Were you still using hormonal contraceptives about two years ago?
   ○ yes
   ○ no
   ○ don’t know
35b. In total, how long did you take these hormonal contraceptives? If you started and stopped and then started again, please count only the time you were taking these contraceptives.

____ ____ number of years  
○ don’t know

36. Have you had a menstrual period in the last 12 months? Please include only menstrual bleeding, not bleeding that results from hormone replacement therapy (HRT) or progestogens, progestins or withdrawal bleeding.

○ yes → please go to #42
○ no
○ don’t know → please go to #42
Have your periods stopped permanently or only temporarily due to pregnancy, breastfeeding, or other conditions?

○ permanently
○ temporarily → please go to #42

37. How old were you when your periods stopped permanently?

age they stopped ____ ____ or
year they stopped ____ ____ ____ ____
○ don’t know

38. Why did your menstrual periods stop permanently? Please tick all that apply.

○ natural menopause
○ surgery
○ radiation or chemotherapy → please go to #39
○ other reason 
please specify: ____________________
→ please go to #40
○ don’t know

39. If you had radiation or chemotherapy, when did you first have it?

○ had radiation or chemotherapy age when this was given ____ ____ or year when this was given ____ ____ ____ ____
○ don’t know
○ never had radiation or chemotherapy

40. If your periods stopped permanently for any reason other than surgery, radiation or chemotherapy, when did this occur?

○ other reason
Please specify: ____________________
age when occurred ____ ____ or
year when occurred ____ ____ ____ ____

41. Hysterectomy (only the uterus or womb removed)

○ yes
○ no
○ don’t know

41a. Hysterectomy with one ovary or part of an ovary removed.

○ yes
○ no
○ don’t know

41b. Hysterectomy with both ovaries removed

○ yes
○ no
○ don’t know

41c. One ovary removed completely or partly without hysterectomy.

○ yes
○ no
○ don’t know

42a. Were you still having menstrual bleeding and any medication (such as Lupron, or Depo-Provera) when you first took these hormones?

○ yes
○ no
○ don’t know

42b. Were you ever prescribed either an estrogen-only pill or patch (such as Premarin) for hormone replacement therapy?

○ yes → Please go to #44
○ no → Please go to #44
○ don’t know

43. Have you ever taken tamoxifen, raloxifene, or other anti-estrogen medication (such as Lupron, or Depo-Provera) for breast cancer treatment?

○ yes → Please go to #44
○ no
○ don’t know

44. Possibly – I have participated in a clinical trial for tamoxifen or other anti-estrogen medication.

...
41d. Both ovaries removed without hysterectomy.
   - yes
   - no
   - don’t know
   - age when removed ______ or
   - year when removed ______
   - don’t know

42. Doctors prescribe hormone replacement therapy for many reasons, including menopausal symptoms, surgical removal of the ovaries, osteoporosis, and heart disease prevention. (Menopausal symptoms include hot flashes, sweating, and depression.) Have you ever taken hormone replacement therapy prescribed by a doctor and in the form of a pill or a patch?
   - Please do not include hormone therapy that was prescribed for birth control, infertility, hormone therapy delivered by injections, vaginal creams or vaginal suppositories, or herbal or soy products.
   - yes
   - no → Please go to #43
   - don’t know → Please go to #43

42c. Doctors prescribe hormone replacement therapy for many reasons, including menopausal symptoms, surgical removal of the ovaries, osteoporosis, and heart disease prevention. (Menopausal symptoms include hot flashes, sweating, and depression.) Have you ever taken hormone replacement therapy prescribed by a doctor and in the form of a pill or a patch?
   - Please do not include hormone therapy that was prescribed for birth control, infertility, hormone therapy delivered by injections, vaginal creams or vaginal suppositories, or herbal or soy products.
   - yes
   - no → Please go to #43
   - don’t know → Please go to #43

42e. Progesterone or progestin is frequently prescribed by doctors together with estrogen for hormone replacement therapy. One common brand name is Provera. Another one is Prometrium. Have you ever taken progesterone or progestin together with estrogens for hormone replacement therapy?
   - yes
   - no → Please go to #43
   - don’t know → Please go to #43

42f. Were you still using progesterone or progestin medication about two years ago?
   - yes
   - no
   - don’t know

42g. In total, how long did you take progesterone or progestin medication together with estrogens? If you started and stopped and then started again, please count only the time you were taking this medication.
   - ______ number of months or
   - ______ number of years
   - don’t know

43a. How old were you when you first took tamoxifen, raloxifene or other anti-estrogen medication?
   - don’t know

43b. Were you still taking tamoxifen, raloxifene or other anti-estrogen medication about two years ago?
   - don’t know

43c. In total, how long did you take tamoxifen, raloxifene or other anti-estrogen medication? If you started and stopped and then started again, please count only the time you were taking this medication.
   - ______ number of months or
   - ______ number of years
   - don’t know
Diet

44. **About two years** ago, on average, how often did you eat a piece or serving of **fruit**?
   (A serving of fruit is: 1 medium-sized fresh fruit; ½ cup of chopped, cooked or canned fruit; ¼ cup of dried fruit; 6 ounces of fruit juice (50%-100% pure juice).) Please choose one of the following.
   - ____ ____ servings per day or
   - ____ ____ servings per week or
   - ____ ____ servings per month
   ○ don’t know

45. **About two years** ago, on average, how often did you eat a serving of **vegetables**? Please include green salads, beans, lentils, etc., and potatoes (not packaged potato chips).
   (A serving of vegetables is: 1 cup raw leafy vegetables; ½ cup of other vegetables, cooked or shopped raw; 6 ounces of vegetable juice.) Please choose one of the following.
   - ____ ____ servings per day or
   - ____ ____ servings per week or
   - ____ ____ servings per month
   ○ don’t know

46. **About two years** ago, on average, how often did you eat a serving of **red meat** (not chicken or fish)?
   (A serving of red meat is: 2-3 ounces of red meat (a piece of meat about the size of a deck of cards). Red meats include: beef, steak, hamburger, prime rib, ribs, beef hot dogs, beef-based processed meat, veal, pork, bacon, pork sausage, ham, lamb, venison.) Please choose one of the following.
   - ____ ____ servings per day or
   - ____ ____ servings per week or
   - ____ ____ servings per month
   ○ didn’t eat red meat ➔ Please go to #47
   ○ don’t know

46a. **About two years** ago, on average, how often did you eat a serving of **red meat** that was cooked by broiling, grilling, barbecuing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.
   - ____ ____ servings per day or
   - ____ ____ servings per week or
   - ____ ____ servings per month
   ○ didn’t eat red meat that was cooked by these methods ➔ Please go to #47
   ○ don’t know
46b. On average, when you ate **red meat** cooked by these methods, which of the following best describes its appearance?

<table>
<thead>
<tr>
<th>Outside appearance</th>
<th>Inside appearance (how well done it was)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ lightly browned</td>
<td>○ red (rare)</td>
</tr>
<tr>
<td>○ medium browned</td>
<td>○ pink (medium)</td>
</tr>
<tr>
<td>○ heavily browned or blackened</td>
<td>○ brown (well-done)</td>
</tr>
<tr>
<td>○ don't know</td>
<td>○ don't know</td>
</tr>
</tbody>
</table>

47. **About two years** ago, on average, how often did you eat a serving of **chicken**? Please do not include turkey or any other bird.

(A serving of chicken is: 2-3 ounces of chicken meat; 1 drumstick; 1 thigh; half a breast; 2 wings; 3 nuggets.) Please choose one of the following.

- ___ ___ servings per day or
- ___ ___ servings per week or
- ___ ___ servings per month

○ didn't eat chicken ➔ Please go to #48

○ don't know

47a. **About two years** ago, on average, how often did you eat a serving of **chicken** that was cooked by broiling, grilling, barbecuing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.

- ___ ___ servings per day or
- ___ ___ servings per week or
- ___ ___ servings per month

○ didn't eat chicken that was cooked by these methods ➔ Please go to #48

○ don't know

47b. On average, when you ate **chicken** cooked by these methods, which of the following best describes its appearance?

<table>
<thead>
<tr>
<th>Outside appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ lightly browned</td>
</tr>
<tr>
<td>○ medium browned</td>
</tr>
<tr>
<td>○ heavily browned or blackened</td>
</tr>
<tr>
<td>○ don't know</td>
</tr>
</tbody>
</table>
Physical Activity
We would like you to think back to when you were in your 20s and remember the physical activities you participated in then.

48. In your 20s, did you participate regularly in physical activity for a total of at least 30 minutes a week?

- [ ] yes ➔ Please describe your activities below
- [ ] no ➔ Please go to #49
- [ ] don’t know ➔ Please go to #49

<table>
<thead>
<tr>
<th>Activity</th>
<th>For how many years?</th>
<th>During those years, for how many months per year?</th>
<th>During those months, on average, for how many minutes or hours per week?</th>
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</thead>
<tbody>
<tr>
<td>Heavy occupational work</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>[ ] no</td>
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<tr>
<td>Heavy household or yard work</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<tr>
<td>(examples: using a non-power mower, scrubbing floors)</td>
<td>[ ] no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>[ ] no</td>
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<td></td>
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<tr>
<td>Jogging (running slower than a mile in 10 minutes)</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>[ ] no</td>
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<tr>
<td>Running (running faster than a mile in 10 minutes)</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
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<td></td>
<td>[ ] no</td>
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<tr>
<td>Bicycling (including using an exercise bicycle)</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td>[ ] no</td>
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<tr>
<td>Swimming laps</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>[ ] no</td>
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<tr>
<td>Tennis, squash racquetball</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>[ ] no</td>
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<tr>
<td>Calisthenics, aerobics, vigorous dance, lifting weights</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
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<td>[ ] no</td>
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<tr>
<td>Football, soccer rugby, basketball</td>
<td>[ ] yes ➔ ___ years</td>
<td>___ months</td>
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<td></td>
<td>[ ] no</td>
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</tbody>
</table>
In your 20s, did you do any other additional recreational strenuous activities? Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, sledding or tobogganing, water-skiing.

- ○ yes
- ○ no → Please go to #49
- ○ don’t know → Please go to #49

<table>
<thead>
<tr>
<th>Activity</th>
<th>For how many years?</th>
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<td>→ __ __ years</td>
<td>__ __ months</td>
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</tr>
</tbody>
</table>

49. When you were in your 20s, what was your usual occupation? (We mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife or being unemployed.)

- ○ don’t know

If you are younger than age 31, please go to the next section (Alcohol consumption) on page 25. Otherwise, please continue with #50.
50. **In your 30s and 40s, did you participate regularly in physical activity for a total of at least 30 minutes a week?**

- [ ] yes → Please describe your activities below
- [ ] no → please go to #51
- [ ] don’t know → Please go to #51

<table>
<thead>
<tr>
<th>Activity</th>
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<tr>
<td>Walking</td>
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<tr>
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<tr>
<td>Swimming laps</td>
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<tr>
<td>yes</td>
<td>___ ___ years</td>
<td>___ ___ months</td>
<td>___ ___ minutes per week or ___ ___ hours per week</td>
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<tr>
<td>no</td>
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<tr>
<td>Tennis, squash, racquetball</td>
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<tr>
<td>yes</td>
<td>___ ___ years</td>
<td>___ ___ months</td>
<td>___ ___ minutes per week or ___ ___ hours per week</td>
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<td>no</td>
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<tr>
<td>Aerobics, vigorous dance, lifting weights</td>
<td>___ ___ years</td>
<td>___ ___ months</td>
<td>___ ___ minutes per week or ___ ___ hours per week</td>
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<tr>
<td>yes</td>
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<tr>
<td>no</td>
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<tr>
<td>Football, soccer, rugby, basketball</td>
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<tr>
<td>yes</td>
<td>___ ___ years</td>
<td>___ ___ months</td>
<td>___ ___ minutes per week or ___ ___ hours per week</td>
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<tr>
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</tbody>
</table>
In your 30s and 40s, did you do any other additional recreational strenuous activities? Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, sledding or tobogganin, water-skiing.

- yes
- no → Please go to #51
- don’t know → Please go to #51

<table>
<thead>
<tr>
<th>Activity</th>
<th>For how many years?</th>
<th>During those years, For how many months per year?</th>
<th>During those months, on average, for how many minutes or hours per week?</th>
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</table>

51. When you were in your 30s and 40s, what was your usual occupation? (We mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife or being unemployed.)

- don’t know

If you are younger than age 51, please go to the next section (Alcohol Consumption) on page 25. Otherwise, please continue with #52.
52. Since you turned 50, did you participate regularly in physical activity for a total of at least 30 minutes a week?

☐ yes → Please describe your activities below
☐ no → Please go to #53
☐ don’t know → Please go to #53

<table>
<thead>
<tr>
<th>Activity</th>
<th>For how many years?</th>
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<tbody>
<tr>
<td>Heavy occupational work</td>
<td></td>
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<tr>
<td>Heavy household or yard work (examples: using a non-power mower, scrubbing floors)</td>
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<td></td>
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</tr>
<tr>
<td>Walking</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
</tr>
<tr>
<td></td>
<td>☐ no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jogging (running slower than a mile in 10 minutes)</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<tr>
<td></td>
<td>☐ no</td>
<td></td>
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<tr>
<td>Running (running faster than a mile in 10 minutes)</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>☐ no</td>
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<tr>
<td>Bicycling (including using an exercise bicycle)</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>☐ no</td>
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<tr>
<td>Swimming laps</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>☐ no</td>
<td></td>
<td></td>
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<tr>
<td>Tennis, squash, racquetball</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>☐ no</td>
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<tr>
<td>Calisthenics, aerobics, vigorous dance, lifting weights</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
<td>___ minutes per week or ___ hours per week</td>
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<td></td>
<td>☐ no</td>
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<tr>
<td>Football, soccer, rugby, basketball</td>
<td>☐ yes → ___ years</td>
<td>___ months</td>
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<tr>
<td></td>
<td>☐ no</td>
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</tbody>
</table>
Since you turned 50, did you do any other additional strenuous activities? Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, sledding or tobogganing, water-skiing.

- yes
- no → Please go to #53
- don’t know → Please go to #53

<table>
<thead>
<tr>
<th>Activity Please specify</th>
<th>For how many years?</th>
<th>During those years, for how many months per year?</th>
<th>During those months, on average, for how many minutes or hours per week?</th>
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</tr>
</tbody>
</table>

53. Since you turned 50, what was your usual occupation? (We mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife or being unemployed.)

__________________________________________________________________________ occupation

- don’t know
Alcohol Consumption

We would like you to think back to when you were in your 20s.

54. **In your 20s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer?** Please describe your consumption below.

<table>
<thead>
<tr>
<th>Alcohol Type</th>
<th>For how many years?</th>
<th>During those years, how much did you typically consume?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer, hard cider (at least 3% alcohol)</td>
<td>〇 yes ➔ ___ ___ years consumed</td>
<td>〇 ___ ___ number of 12 ounce cans or bottles per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 no</td>
<td>〇 per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 don’t know</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>〇 yes ➔ ___ ___ years consumed</td>
<td>〇 ___ ___ number of 4 ounce glasses or wine per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 no</td>
<td>〇 per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 don’t know</td>
<td></td>
</tr>
<tr>
<td>Sake, sherry, port</td>
<td>〇 yes ➔ ___ ___ years consumed</td>
<td>〇 ___ ___ number of 1 ounce servings per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 no</td>
<td>〇 per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 don’t know</td>
<td></td>
</tr>
<tr>
<td>Spirits, liquor mixed drinks, brandy, liqueurs</td>
<td>〇 yes ➔ ___ ___ years consumed</td>
<td>〇 ___ ___ number of 1 ounce shots liquor or spirits per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 no</td>
<td>〇 per day per week don’t know</td>
</tr>
<tr>
<td></td>
<td>〇 don’t know</td>
<td></td>
</tr>
</tbody>
</table>

55. When you were in your 20s, how many years in total did you consume at least one alcoholic beverage (beer, wine or hard liquor) a week?

___ ___ years consumed

〇 never (did not regularly consume at least one alcoholic beverage per week)

56. Approximately, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ number of alcoholic beverages a week

〇 never (did not regularly consume at least one alcoholic beverage per week)

*If you are younger than age 31, please go to the next section (Smoking) on page 28. Otherwise, please continue with #57.*
57. In your 30s and 40s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

<table>
<thead>
<tr>
<th>For how many years?</th>
<th>During those years, how much did you typically consume?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer, hard cider (at least 3% alcohol)</td>
<td>○ yes → ___ years consumed ___ number of 12 ounce cans or bottles per day per week don’t know</td>
</tr>
<tr>
<td>○ no</td>
<td></td>
</tr>
<tr>
<td>○ don’t know</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>○ yes → ___ years consumed ___ number of 4 ounce glasses or wine per day per week don’t know</td>
</tr>
<tr>
<td>○ no</td>
<td></td>
</tr>
<tr>
<td>○ don’t know</td>
<td></td>
</tr>
<tr>
<td>Sake, sherry, port</td>
<td>○ yes → ___ years consumed ___ number of 1 ounce servings per day per week don’t know</td>
</tr>
<tr>
<td>○ no</td>
<td></td>
</tr>
<tr>
<td>○ don’t know</td>
<td></td>
</tr>
<tr>
<td>Spirits, liquor, mixed drinks, brandy, liqueurs</td>
<td>○ yes → ___ years consumed ___ number of 1 ounce shots per day per week don’t know</td>
</tr>
<tr>
<td>○ no</td>
<td></td>
</tr>
<tr>
<td>○ don’t know</td>
<td></td>
</tr>
</tbody>
</table>

58. When you were in your 30s and 40s, how many years in total did you consume at least one alcoholic beverage (beer, wine or hard liquor) a week?

___ ___ years consumed

○ never (did not regularly consume at least one alcoholic beverage per week)

59. Approximately, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ number of alcoholic beverages a week

○ never (did not regularly consume at least one alcoholic beverage per week)

If you are younger than age 51, please go to the next section (Smoking) on page 28. Otherwise, please continue with #60.
Now, please think back to **since you turned 50**.

60. **Since you turned 50**, did you ever consume any alcoholic beverages at least **once a week for 6 months or longer**? Please describe your consumption below.

<table>
<thead>
<tr>
<th></th>
<th>For how many years?</th>
<th>During those years, how much did you typically consume?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer, hard cider (at least 3% alcohol)</td>
<td>☐ yes ➔ │ ☠ years consumed │ ☠ number of 12 ounce cans or bottles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☐ no</td>
<td>☠ per day</td>
</tr>
<tr>
<td></td>
<td>☠ don’t know</td>
<td>☠ per week</td>
</tr>
<tr>
<td></td>
<td>☠ don’t know</td>
<td>☠ don’t know</td>
</tr>
</tbody>
</table>

| Wine     | ☐ yes ➔ │ ☠ years consumed │ ☠ number of 4 ounce glasses or wine |
|          | ☐ no               | ☠ per day                                               |
|          | ☠ don’t know       | ☠ per week                                              |
|          | ☠ don’t know       | ☠ don’t know                                            |

| Sake, sherry, port | ☐ yes ➔ │ ☠ years consumed │ ☠ number of 1 ounce servings |
|                    | ☐ no               | ☠ per day                                               |
|                    | ☠ don’t know       | ☠ per week                                              |
|                    | ☠ don’t know       | ☠ don’t know                                            |

| Spirits, liquor mixed drinks, brandy, liqueurs | ☐ yes ➔ │ ☠ years consumed │ ☠ number of 1 ounce shots liquor or spirits |
|                                               | ☐ no               | ☠ per day                                               |
|                                               | ☠ don’t know       | ☠ per week                                              |
|                                               | ☠ don’t know       | ☠ don’t know                                            |

61. When you were in your 50s, how many years **in total** did you consume **at least one alcoholic beverage (beer, wine or hard liquor) a week**?

  ☠ years consumed

  ☐ never (did not regularly consume at least one alcoholic beverage per week)

62. Approximately, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

  ☠ number of alcoholic beverages a week

  ☐ never (did not regularly consume at least one alcoholic beverage per week)
**Smoking**

63. Have you ever smoked at least one cigarette a day for at least 3 months or longer?
   - ○ yes
   - ○ no → Please go to #64
   - ○ don’t know → Please go to #64

63a. When did you **first** start smoking at least one cigarette a day?
   - age at **first** use ____ ____ or
   - year of **first** use ____ ____ ____ __
   - ○ don’t know

63b. During periods when you smoked regularly, how many cigarettes did you typically smoke in a day?
   - ____ ____ ____ cigarettes per day
   - ○ don’t know

63c. **About two years** ago, were you still smoking at least one cigarette a day?
   - ○ yes
   - ○ no
   - ○ don’t know

63d. Do you still smoke at least one cigarette a day?
   - ○ yes → Please go to #63f
   - ○ no → Please go to #63e
   - ○ don’t know

63e. When did you stop smoking at least one cigarette a day (we mean stop smoking permanently)?
   - age at last use ____ ____ or
   - year of last use ____ ____ ____ ____
   - ○ don’t know

63f. How many years, in total, did you smoke at least one cigarette a day for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)
   - ____ ____ total number of years
   - ○ don’t know

---

64. Have you ever smoked at least one **cigar** a month for at least 3 months?
   - ○ yes
   - ○ no → Please go to #65
   - ○ don’t know → Please go to #65

64a. When did you **first** start smoking at least one cigar a month?
   - age at **first** use ____ ____ or
   - year of **first** use ____ ____ ____ __
   - ○ don’t know

64b. During periods when you smoked regularly, how many cigars did you typically smoke in a month?
   - ____ ____ ____ cigars per month
   - ○ don’t know

64c. **About two years** ago, were you still smoking at least one cigar a month?
   - ○ yes
   - ○ no
   - ○ don’t know

64d. Do you still smoke at least one cigar a month?
   - ○ yes → Please go to #64f
   - ○ no → Please go to #64e
   - ○ don’t know

64e. When did you stop smoking at least one cigar a month (we mean stop smoking permanently)?
   - age at last use ____ ____ or
   - year of last use ____ ____ ____ ____
   - ○ don’t know

64f. How many years, in total, did you smoke at least one cigar a month for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)
   - ____ ____ total number of years
   - ○ don’t know
65. Have you ever smoked at least one pipe a month for at least 3 months?
   - yes
   - no  → Please go to #66
   - don’t know  → Please go to #66

65a. When did you first start smoking at least one pipe a month?
   - age at first use _____ or
   - year of first use
   - ____ ____
   - don’t know

65b. During periods when you smoked regularly, how many pipes did you typically smoke in a month?
   - ____ ____ ____ pipes per month
   - don’t know

65c. About two years ago, were you still smoking at least one pipe a month?
   - yes
   - no
   - don’t know

65d. Do you still smoke at least one pipe a month?
   - yes  → Please go to #65f
   - no  → Please go to #65e
   - don’t know

65e. When did you stop smoking at least one pipe a month (we mean stop smoking permanently)?
   - age at last use _____ or
   - year of last use
   - ____ ____
   - don’t know

65f. How many years, in total, did you smoke at least one pipe a month for 3 months or longer? If you have stopped and restarted at least once, count only the time when you were smoking.
   - ____ ____ total number of years
   - don’t know

---

66. About how tall are you, without your shoes on?
   - _____ feet _____ inches
   - or
   - _____ _____ centimetres
   - don’t know

67. How much did you weigh about two years ago?
   - _____ pounds
   - or
   - _____ kilograms
   - don’t know

68. How much did you weigh when you were about 20 years old?
   - _____ pounds
   - or
   - _____ kilograms
   - don’t know

---

Additional Information

69. Previous to this study, have you and your relatives ever taken part in any family health studies?
   - yes
   - no
   - don’t know
**Background Information**

70. What is the highest level of education that you completed?

- ○ less than 8 years
- ○ 8 to 11 years
- ○ high school graduate
- ○ vocational or technical school
- ○ some college or university
- ○ bachelor’s degree
- ○ graduate degree
- ○ don’t know

71. Country of birth sometimes affects disease risk. Please fill in country of birth for **yourself, your parents and your grandparents.**

In addition, scientists have found that some genetic traits are more common or less common among Jewish people of different ethnic backgrounds. Please answer the questions about Jewish descent for each person.

<table>
<thead>
<tr>
<th>Country of birth</th>
<th>Is this person of Jewish descent?</th>
<th>Ashkenazi (East European)</th>
<th>Sephardic</th>
<th>Other Jewish descent</th>
<th>don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your mother</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your father</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your mother’s mother</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your mother’s father</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your father’s mother</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your father’s father</td>
<td>○ yes ➔</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td></td>
<td>○ no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>○ don’t know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
72. How many years have you lived in Canada?

- all my life
- __ __ number of years
- don’t know

73. Ethnicity and race sometimes affect disease risk. Scientists have found that some genetic traits are more common or less common among people of different backgrounds. We would like to know if this is true for genes associated with colorectal cancer.

Please fill in the background for yourself, your parents and your grandparents. Please tick all that apply.

<table>
<thead>
<tr>
<th>Background</th>
<th>You</th>
<th>Your mother</th>
<th>Your father</th>
<th>Your mother’s mother</th>
<th>Your mother’s father</th>
<th>Your father’s mother</th>
<th>Your father’s father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black, from Africa</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Black, from the Caribbean (e.g. Trinidad, Jamaica, Haiti)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Black, from North America</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Black, other</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>White</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>First Nations (e.g. Indian, Inuit)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>North African (e.g. Egyptian)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Middle Eastern (e.g. Iranian)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Filipino</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Japanese</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Korean</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Chinese</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other South East Asian (e.g. Vietnamese)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>South Asian (e.g. East Indian, Pakistani)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>other: please specify</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>don’t know</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
74. Which of the following categories best describes your total annual household income about two years ago?

- no income
- less than $6,000
- $6,000 - $11,999
- $12,000 - $19,999
- $20,000 - $29,999
- $30,000 - $39,999
- $40,000 - $49,999
- $50,000 - $59,999
- $60,000 - $69,999
- $70,000 - $79,999
- $80,000 or more
- don’t know

75. In case we need to contact you in the future and you have moved, could we have the name of someone who is not living with you to whom we might write or call for your new address?

Name of relative or friend: ________________________________________________________________

His or her address: _______________________________________________________________________
_______________________________________________________________________________________

His or her telephone number:

home: ( ___ ___ ___ ) ___ ___ ___ - ___ ___ ___ ___

business: ( ___ ___ ___ ) ___ ___ ___ - ___ ___ ___ ___

fax: ( ___ ___ ___ ) ___ ___ ___ - ___ ___ ___ ___

e-mail: ______________________________________________

we will keep this information confidential
Appendix D: Ethics approval letter from University of Toronto Ethics Board for study 2 “Effect of *H. pylori* infection on bioavailability of vitamins C and E”.
UNIVERSITY OF TORONTO  
Office of the Vice-President, Research and Associate Provost  
Ethics Review Office  

PROTOCOL REFERENCE #15344  
October 5, 2005  

Ms. F. Naja  
30 Claridge St, W., Apt. 1009  
Toronto, ON M4Y 1R5  

Dr. N. Kreiger  
Director of Research  
Senior Epidemiologist  
Cancer Care Ontario  
620 University Ave., 15th Floor  
Toronto, ON MSG 2L7  

Dear Dr. Kreiger & Ms. Naja:  

Re: Your research protocol entitled, “Effect of Helicobacter pylori on the systemic availability of vitamins C and E” (Revised October 3, 2005) by Dr. N. Kreiger (supervisor), Ms. F. Naja (PhD candidate) 

ETHICS APPROVAL  
Original Approval Date: October 5, 2005  
Expiry Date: October 4, 2006  

We are writing to advise you that the Health Sciences II Research Ethics Board has granted approval to the above-named research study, for a period of one year. Ongoing projects must be renewed prior to the expiry date. Your ethics protocol approval is valid for a period of 1 year. It is the responsibility of the investigator to maintain a valid approval throughout the duration of the research activity, and to report to the Ethics Review Office of its completion. Annual Renewal of Ethics Approval forms and Study Completion Report forms can be found at http://www.rrr.utoronto.ca/ethics_hsmaterials.html. Consequences of expired ethics protocol approvals may include the freezing of funds and/or refusal to review new ethics protocol submissions.  

The following documents have been approved for use in this study: Information Sheet/Consent Form, Demographic & Medical History Patient Information Sheet, Dietary Intake Record and Recruitment Flyer. Participants should receive a copy of their consent form.  

During the course of the research, any significant deviations from the approved protocol (that is, any deviation which would lead to an increase in risk or a decrease in benefit to participants) and/or any unanticipated developments within the research should be brought to the attention of the Ethics Review Unit.  

Best wishes for the successful completion of your project. 

Yours sincerely, 

Marianna Richardson  
Ethics Review Coordinator  

Simcoe Hall 27 King’s College Circle Toronto Ontario M5S 1A1  
Telephone 416/978-3165  Fax 416/946-5763  email: ethics.review@utoronto.ca
Appendix E: Ethics approval letter from University Health Network Ethics Board for study 2 “Effect of H. pylori infection on bioavailability of vitamins C and E”.
Appendix F: Advertisement for subjects’ recruitment for study 2.

Research Volunteers Needed For a Study of Vitamin Supplements

Males and females (non smokers) aged 18-45 are needed to:

• Take vitamin E and C supplements for 4 weeks
• Give a blood sample before and after supplementation
• Complete two 7-day food records & a 2-page questionnaire

In order to participate, you must be born outside Canada (except North America, Western Europe and China) and immigrated to Canada at 10 years of age or older

** $50 compensation will be provided

For further information contact Farah at 416-971-9800, ext 3216 or farah.naja@cancercare.on.ca
Appendix G: Subjects consent form for study 2.

Subjects Information Sheet & Consent Form

STUDY TITLE: Effect of *H. pylori* infection on the systemic availability of vitamins C and E

PRINCIPAL INVESTIGATOR: Farah Naja, PhD candidate, Nutritional Sciences, U of T.
Tel: (416) 971 9800 ext. 3216

CO-INVESTIGATOR: Dr. Nancy Kreiger, Dr Joanne Allard, Nutritional Sciences, U of T

SPONSOR: Canadian Cancer Etiology Research Network. Jamieson Laboratories

Introduction
Dietary antioxidants including vitamins C and E are important in the body defense against harmful substances such as reactive oxygen and nitrogen species. These substances are associated with an increased risk of cancer. *H. pylori* infection increases production of these substances. The proposed study aims at testing the possibility that *H. pylori* infection may decrease the availability of dietary antioxidants.

You are invited to participate in this research study if you are:
- 18-45 years old
- Born outside Canada (except North America, Western Europe and China and immigrated) to Canada at 10 years or older
- Non smoker
- Average weight (weight Kg/(ht)^2m between 18 and 25)
- Have never received treatment for *H. pylori* infection
- Have not had surgery in the stomach
- Not currently taking antioxidants supplements, proton pump inhibitors (PPI).
- Not training in an athletic team
- Drinking no more than three servings of alcohol per day

Purpose
Vitamins E and C play an important role in the body’s defense mechanism against oxidants. The purpose of this study is to assess whether *H. pylori* neutralizes the effect of the dietary antioxidants, rendering them less available to perform their protective role.

Procedures
This study is a controlled trial. You will belong to either group 1 or group 2 depending on your status of *H. pylori* infection. Both groups will consume 2 pills of vitamin supplements daily for a period of 4 weeks. The pills contain 400 IU of vitamin E and 500 mg of vitamin C. During this study, there will be 3 visits to the gastroenterology clinic at the Toronto General Hospital. Each visit will take about 30-40 minutes.
Initial visit
The study coordinator will explain the study and answer your questions. You will then take the urea breath test, which will determine your H. pylori infection status. This test requires you to blow first in an empty tube, ingest a solution of urea, wait for thirty minutes and then blow again in another empty tube. The study coordinator will call you to inform you about your H. pylori infection status. If there is a vacancy in the group that you belong to (H. pylori +ve or H. pylori –ve), the study coordinator will ask you to continue the study. If not, then she will offer you $10 as compensation and will dismiss you from the rest of the study.

During this visit, the project coordinator will instruct you on how to complete a 7 day food record. Should you continue the study, you will be to bring the 7 day food record in the next visit. The records belong to the 7 days prior to visit 2. For the subsequent visit, the study coordinator may call you in advance to remind you of your appointment and to come in a fasting state (did not eat or drink anything in the morning before your visit).

2nd visit:
The study coordinator will measure your body weight, height, waist and hip circumferences. She will collect the 7 day food records.
A licensed phlebotomist will draw blood from your arm (4 Tbsps). This blood sample will be used to assess the levels of vitamin E and C in your blood. She will ask you to take a daily vitamin E and C supplement (in the form of tablets). She will give you a 4-week supply of the pills and ask you to bring your leftover pills to the next visit. In addition she will ask you to complete the second set of 7 day food records belonging to the week prior to the next visit.

3rd visit:
You will bring back your 7-day food intake record and any left over pills. The laboratory technician will take blood from your arm (4 Tbsp) in order to re-assess the levels of vitamin C and E.
Participation and Withdrawal
Your participation in this study is voluntary. You can choose not to participate or you may withdraw at any time.

Risks
There may be some minor discomfort or bruising associated with the blood sample. There are no side effects reported with vitamin C and E supplements at the doses used in this study. The urea breath test is safe for adults, children and women of childbearing age.

Benefits
You will have an H. pylori infection test. Under normal circumstances this test is not covered by OHIP. If you are H. pylori positive, the study coordinator will refer to your family physician for treatment, which is a simple course of antibiotics.
If you wish, we can also send you the result of the study once obtained.

Remuneration
You will receive $50.00 as compensation for your time and traveling once you complete the study. You will not pay for any test during the research process. If you are dismissed after the first visit because of the group you belong to (H. pylori +ve or –ve) has no vacancy, you will receive $10 as compensation. If you leave the study prematurely, you will be reimbursed ($25) for the time you have spent in the study.

Confidentiality
The investigators are responsible to ensure that all information obtained during the study is in strict confidence. They will assign a random identification number to your file and store it in a locked cabinet. They will protect electronic files by passwords. Only the investigators involved in the trial will have access to your data. Publications and presentations will not include any names or identifying information

Contact Person
If you have any questions about the study or experience any side effects, please contact Farah Naja (Study Coordinator) at 9719800 ext.3216.
If you have any concern about your rights as a research subjects, please contact Jill Parsons, Research Ethics Officer, jc.parsons@utoronto.ca or 416-946-5806.

Consent
I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties.
I consent to take part in the study with the understanding that I may withdraw at any time without affecting my medical care. I have received a signed copy of this consent form. I voluntarily consent to participate in this study. ________________

Subject’s Name (Please Print) ____________________ Subject’s Signature
Appendix H: Demography questionnaire for study 2.

**Effect of *H. pylori* infection on the systemic availability of dietary vitamin C and E**

Visit 1: at Toronto General Hospital – page 1

Visit Day (dd/mm/yy) ___/___/___

Subject ID: ______________________

INCLUSION CRITERIA (If the answer to any of these is NO, subject is not eligible)

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Age 18 – 30 years old</td>
<td></td>
</tr>
<tr>
<td>ii) Body Mass Index (BMI (Wt(Kg)/Ht’m) between 18 and 25</td>
<td></td>
</tr>
<tr>
<td>iv) Immigrant who came to Canada at 10 years or older</td>
<td></td>
</tr>
</tbody>
</table>

EXCLUSION CRITERIA (If the answer to any of these is YES, subject is not eligible)

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Smoker</td>
<td></td>
</tr>
<tr>
<td>ii) Receive previous treatment to <em>H. pylori</em> infection</td>
<td></td>
</tr>
<tr>
<td>iii) Have undergone surgery for partial or total gastrectomy</td>
<td></td>
</tr>
<tr>
<td>iv) Currently taking antioxidants supplements</td>
<td></td>
</tr>
<tr>
<td>v) Train with an athletic team</td>
<td></td>
</tr>
<tr>
<td>vi) Drink 3 or more servings of alcohol per day</td>
<td></td>
</tr>
</tbody>
</table>

Sex:      M      F
Date Of Birth: _____/_____/_____
(dd/mm/yy)

Marital Status: Currently married or living as married
Separated
Divorced
Widowed
Single or never married

Number of siblings:
Less or equal to 2
2 to 4
>4

Sharing bed with siblings during childhood: Yes  No
Taking more medicines than siblings during childhood: Yes  No
Taking more antibiotics than siblings during childhood: Yes  No

Number of rooms: _______________
Place of birth: ________________

Please specify the age of migration:
Race:  Black
       White
       Asian
       Other:
       Please specify

Anthropometry
Anthropometric measurements:
Wt: _____________ Kg
Ht: _______________ cm

Medical History
<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal history of peptic ulcer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of peptic ulcer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of gastric cancer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal history of heartburn:</td>
<td></td>
<td></td>
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<tr>
<td>Do you consume alcohol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Amount: ______________
Appendix I: Food records form for study 2

**DIETARY INTAKE RECORD**

**General Information:**

A complete and accurate food record is essential.

Please read instructions before beginning to record your food intake.

Please keep an accurate record of everything you eat and drink for 7 days: 1 day should be on the week-end. Include all snacks, alcoholic beverages, cigarette smoking, and condiments.

- List each food item in as much detail as possible.
- Try to describe the amount of food eaten.
- Include the portion size model for each food item consumed.
- For each food, briefly describe how it was prepared.
- Include brand names where possible.

**Recommendations of 2D Models Used To Describe Foods**

<table>
<thead>
<tr>
<th>Volume Models (A Side)</th>
<th>Weight Models (B Side)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages</td>
<td>Beef, lamb, pork, fish</td>
</tr>
<tr>
<td>Cakes, quiches, pies</td>
<td>Luncheon meats</td>
</tr>
<tr>
<td>Canned goods, vegetables, fruits, fish, etc.</td>
<td>Solid cheese</td>
</tr>
<tr>
<td>Casseroles, stews, potted meats, cottage cheese</td>
<td>Sliced chicken, turkey</td>
</tr>
<tr>
<td>Cereals, beans, nuts, chips</td>
<td>Biscuits, muffins, cakes, some cookies</td>
</tr>
<tr>
<td>Fresh cooked vegetables, fruits</td>
<td></td>
</tr>
<tr>
<td>Frozen cooked vegetables, fruits</td>
<td></td>
</tr>
<tr>
<td>Lettuce and other salad items</td>
<td></td>
</tr>
<tr>
<td>Noodles, macaroni, spaghetti, rice, etc.</td>
<td></td>
</tr>
<tr>
<td>Sauces, soups, gravy, salad dressing, etc.</td>
<td></td>
</tr>
<tr>
<td>Sugar and other condiments</td>
<td></td>
</tr>
</tbody>
</table>

E.g. Beef & Broccoli Stir Fry – should be recorded as:

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Description</th>
<th>No.</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef strips</td>
<td>Lean, stir fried</td>
<td>1.0</td>
<td>A15</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Fresh, stir fried</td>
<td>0.5</td>
<td>A15</td>
</tr>
<tr>
<td>Pea Pods</td>
<td>Fresh, stir fried</td>
<td>0.25</td>
<td>A15</td>
</tr>
<tr>
<td>Beef Sauce</td>
<td>Thick, salty</td>
<td>0.25</td>
<td>A15</td>
</tr>
</tbody>
</table>

**Checklist: Have you included the amount and kind of:**

- Spread on bread, toast, vegetables, rice, etc...
- Milk and/or Lactaid, Lactase, soy milk in cereal and beverages
- Salad dressings; fats, oils in cooking, frying, etc.
- Sugar, jams, jellies, syrups
- Candy, chocolate
- Snack foods such as potato chips, pretzels, etc.
- All fluids/beverages
<table>
<thead>
<tr>
<th>Time</th>
<th>Food Item</th>
<th>Description (Frozen, fried, broiled; salted; brand name; etc)</th>
<th>No. of servings</th>
<th>Amount/Model</th>
<th>Multiple/ B side thickness</th>
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Has this been a typical day?  Yes  □  No  □
Appendix J: Helikit™ brochure

**Helikit™** - for the diagnosis of *Helicobacter pylori*

*Helicobacter pylori* (H. pylori) is a bacterium that infects a large proportion of the population. Most individuals infected with H. pylori develop chronic gastritis, which may progress into a duodenal or gastric ulcer. Many ulcer patients develop gastric cancer. Fully 20% to 40% of the North American population and up to 70% of the South East Asian population are reported to be infected with this bacterium. H. pylori eradication may be obtained using antibiotic therapy. However, without proper diagnosis and confirmation of eradication, proper management cannot be achieved.

Isodagnostika has developed a novel, non-invasive, non-radioactive and, convenient breath test kit for the diagnosis of H. pylori called Helikit™. This simple test incorporates Isodagnostika's process and 

\[ ^{13}C \text{ substrates} \] for the analysis of breath samples. The breath sample is then analyzed by an isotope ratio mass spectrometer (IRMS). Helikit™ is also available in a version for analysis by ISOMAX 200™, the innovative infrared point-of-care instrument developed by Isodagnostika that will allow for analysis in a physician's office. Please note that patients are required to fast for four hours prior to taking Helikut™.

Since Helikut™ is non-radioactive, it is safe to be used on children over the age of 6 and during pregnancy. It is simple to perform and can be readily used in the physician's office in a point-of-care environment with the ISOMAX 200™ or in medical laboratories with an IRMS.

Although a number of diagnostic products are available for the diagnosis of H. pylori, Helikut™ is the only test that is non-invasive, non-radioactive, safe and requires only the patients' breath samples and is available in formats for use on either an IRMS or point-of-care format (ISOMAX 200™). This unique test is a research product of Isodagnostika, the leader in breath test analysis, and a company specializing in the use of non-radioactive isotopes.
PyloriTest. Four Simple Steps:

Fast for at least 4 hours before the test.

1. Complete the identification information:
   - Green and yellow identification labels and registration card.

2. Collect first breath sample:
   - Exhale for 4 seconds through the straw into the bottom of the collection tube.
   - Immediately replace cap on tube and tighten.
   - Affix the green label.

3. Drink the solution:
   - Prepare the solution by adding 75 ml. of water to the plastic container and mix
     until completely dissolved, then slowly drink the solution.
   - Wait 30 minutes (no other food or drink during this time).

4. Collect second breath sample:
   - Exhale for 4 seconds through the straw into the bottom of the second collection tube.
   - Immediately replace cap on tube and tighten.
   - Affix yellow label.

Samples with registration card will be sent for analysis.
Refer to package for complete instructions.
Appendix K: Two dimensional chart (portion sizes) used in food records recording for study2

Appendix L: Assays for plasma ascorbic acid, alpha tocopherol, malondialdehyde and thiols (study 2)

Ascorbic acid assay:
Reagents:
- Meta-phosphoric acid: mixture of 35% meta-phosphoric acid (HPO3) with 65% stabilizer as sodium metaphosphate (NaPO3). Store at room temperature. Skin irritant.
- Sulfuric acid concentrate: Store at room temperature. Very irritant.
- 2,4-dinitrophenylhydrazine, FW 198.1, store at room temperature. Inflammable, toxic and carcinogenic.
- Thiourea, FW 76.12, store at room temperature. Toxic and carcinogenic.
- Cupric sulfate pentaydrate, FW 249.7, store at room temperature, Toxic.

Preparation of solutions:
- 5% metaphosphoric acid (5% MPA): dissolve 25 g of MPA in 400 ml of deionized water and dilute to final volume of 500 ml. Store at 4 degrees C for up to 1 week.
- 10% metaphosphoric acid (10% MPA): dissolve 10 g of MPA in 80 ml of deionized water and dilute to final volume of 100 ml. Store at 4 degrees for up to 1 week. If left at room temperature, MPA will undergo molecular rearrangement and loss of assay activity. MPA left at room temperature for longer than 2-3 hours should not be used for the assay.
- 4.5 mol/l sulfuric acid (25% sulfuric acid): with caution, carefully add 250 ml of concentrated sulfuric acid (18 ml/l) to 750 ml of deionized water and mix well. Store at 4 degrees.
- 65% sulfuric acid. With caution add 1300 ml concentrated sulfuric acid (18 mol/l) to 700 of ice-cold deionized water and mix well. Store at 4 degrees.
- 2.2 % 2,4-DNPH: dissolve 1.21 g of 2,4-DNPH in 55 ml of 4.5 mol/l sulfuric acid. Mix well, and then filter until solution is clear. Store at 4 degrees for up to 2 weeks.
-5% Thiourea: dissolve 5 g of thiourea in 80 ml of deionized water. Mix well, and dilute to 100 ml with deionized water. Store at 4 degrees for up to 2-4 weeks.
-1% copper sulfate: dissolve 0.78 g of cupric sulfate-5 hydrate in 40 ml of deionized water. Mix well and dilute to 100 ml with deionized water. Store at 4 degrees for up to 2-4 weeks.
Color reagent. Combine 2,4-DNPH, thiourea and cupric sulfate solutions (20:1:1). During preparation, add thiourea to 2,4-DNPH and mix well prior to adding cupric sulfate. Store at 4 degrees and prepare only enough solution to be used within 4 hours.

Preparations for standards for standard curve:

Stock L- ascorbic acid standard. Weigh out 10-20 mg ascorbic acid powder and prepare 1 mg/ml solution using 5% MPA. Prepare and use daily, store at 4 degrees until discarded.

Add 400 ul of stock standard to 9600 ul of 5% MPA 4 mg/dl
Add 300 ul of stock standard to 9700 ul of 5% MPA 3 mg/dl
Add 200 ul of stock standard to 9800 ul of 5% MPA 2 mg/dl
Add 100 ul of stock standard to 9900 ul of 5% MPA 1 mg/dl
Add 4ul of 1.00 mg/dl standard to 4.0 ml 5% MPA 0.5 mg/dl
Add 2ul of 0.5 mg/dl standard to 2.0 ml 5% MPA 0.25 mg/dl
Add 5 ml of 5% MPA only 0 mg/dl

Vitamin C concentrations in serum/plasma are determined using calculated slope and intercept based on the absorbance readings of L-ascorbic acid standards at 521.0 nm. Seven tests of three standards are used for these calculations. Vitamin C concentrations are derived based on the equation: X= absorbance reading at 521 nm-absorbance intercept/slope.

This method determines total biologically active vitamin concentrations (the sum of Lascorbic acid and dehydro ascorbic acid in biological material) spectrophotometrically using 2,4-DNPH as the chromogen (Bessey et al., 1947) and as modified for use in the NHANES 2 Health and Nutrition Survey.

Preparation of samples:

Take the samples outside the deep freezer for defrost. All samples are analyzed in duplicates and the values reported are the averages of two separate determinations.

Assay procedure

1. Turn on incubator for 28 degrees incubation of samples.
2. Remove extracts from freezer and allow to thaw at room temperature. Used thawed sample immediately upon thawing, do not allow sample to remain at room temperature. If thawed specimen contains protein it must be centrifuged to obtain a clear supernatant for analysis.
3. In duplicate, pipet 800 ul of sample extract or control specimen into a clean test tube.
4. Add 270 ul of color reagent to each sample and mix by vortex.
5. Prepare standards as directed above. For analysis, each standard is prepared in triplicate.
6. After mixing, all tubes should be covered with clear wrap and rubber band and incubated for 20 hour at 28 degrees.
7. After incubation, remove tubes to ice bath for 15 minutes and then add 1300 ul of 65% sulfuric acid to each tube. Mix well by vortex.
8. Allow tubes to stand at room temperatures for 30 minutes before measuring their absorbance levels. The yellow-orange color that develops during this 30 minute incubation is stable for at least 2 hours.
9. Determine absorbance at 521 nm using 5% MPA-65% Sulfuric acid as the analysis blank.

**Alpha tocopherol assay**

Quantitative determination of Alpha tocopherol in plasma is commonly performed by high performance liquid chromatography (HPLC) using a reverse phase column (Teissier et al., 1996). The HPLC (Varian® Canada) apparatus was equipped with a photo-diode array detector (Prostar, model 330), a solvent delivery module (Prostar, model 230), an autosampler (Prostar 410) and an OmniSphere C18 column (Varian®, Canada). The protocol used in the assessment of plasma alpha tocopherol is described by Natta et al., 1988.

The lipid fraction of the plasma was extracted using a hexane/ethanol solution according to the protocol described by Folch et al., 1957. After thawing the samples, 400 ul of the plasma was added to 800 ul of BHT/ethanol solution (35 mg of butylated hydroxyl toluene in 100 ml of HPLC grade ethanol). After mixing for 10 seconds using a vortex, 2 ml of HPLC-grade n-hexane was added. A mechanical shaker was used to mix for 10 minutes. The solution was then centrifuged at 2400 rpm for 10 minutes. After centrifugation, the lipid phase (the upper layer) was carefully transferred into a pre-weighed clean test tube using a pasture pipette. To the lower phase (the water phase) was then added another 1 ml of n-hexane and mixed using mechanical shakers for additional 10 minutes. The solution was then centrifuged at 2400 rpm for 10 minutes. The lipid phase was separated and added to the previously separated lipid solution. The solution was then placed under a flow of nitrogen and allowed to evaporate. After complete evaporation of the liquid, the remaining lipid
residue along with the pre-weighed test tube was weighed. The weight of the lipid fraction was then estimated by subtracting the weight of the empty tube from the weight of the tube with the lipid residue. The lipid residue was then reconstituted with 400 ul of Ether-Methanol solution (90 ml of HPLC grade methanol and 30 ml of stabilizing ether) and 50 ul was injected into the HPLC. The mobile phase consisted of 95% HPLC-Grade Methanol for the first 2.5 minutes and pure 100% HPLC-grade methanol for the remaining of the run. The column temperature was kept at 25 C and the flow rate was at 1.3 ml/min for the first 2.5 minutes and then increased to 1.7 ml/min for the remaining of the run. The retention times for alpha tocopherol was between 6.5-8 minutes at the wavelength of 292 nm.

Standards for dl-rac- alpha-tocopherol acetate (Sigma, Cat #: T3251 were prepared by making a 372 mg/L & 60 mg/L of alpha stock solution in BHT/ethanol mix. Serial dilutions were made and a standard curve was prepared for each by plotting the concentration of each compound vs. peak area of each compound for a given concentration. The samples concentration was then determined using the regression equation derived form the standard curve for each compound.

**Malondialdehyde assay**

Reagents:

Perchloric acid (PCAHClO4), potassium dihydrogen phosphate (KH2PO4), potassium hydroxide (KOH), 2-thiobarbituric acid (2-TBA; C4H4N2O2S), and 1,1,3,3-tetraethoxypropane (TEP; C11H24O4), Fluka, and richloroacetic acid (TCA; CCl3COOH), methanol (CH3OH), and acetonitrile (LiChrosolv for chromatography: CH3CN) was from Merck (Darmstadt, Germany).

Sterile water was used for all the solutions A 40-mM 2-TBA solution was prepared by dissolving 576 mg of 2-TBA in 80 ml of water, and then heating to 55°C for 45 min in a water bath. The solution was cooled to room temperature and filled with water to 100 ml. Buffer: A 50-mM KH2PO4 solution was prepared and adjusted to pH 6.8 with KOH. The buffer was then filtered through a Millipore membrane filter (Millipore, Billerica, MA) with a pore size of 0.45 μm.
HPLC mobile phase consisted of 72:17:11 (by vol) KH2PO4– methanol–acetonitrile. The mobile phase was degassed before use both by helium and a Shimadzu GT-154 vacuum degasser. Reagent kit for HPLC analysis of malondialdehyde in plasma/serum was obtained from Chromsystems Instruments and Chemicals (Cat. no. 67,000, Chromsystems Instruments and Chemicals GmbH, Munich, Germany).

The HPLC (Varian®, Canada) apparatus was equipped with a photo-diode array detector (Prostar, model 330), a solvent delivery module (Prostar, model 230), an auto-sampler (Prostar 410) and an OmniSphere C18 column (Varian®, Canada).

Protocol:

Plasma/serum or standard/blank (50 μL) was mixed with PCA (0.1125 N, 150 μL) and TBA (40 mM, 150 μL) in a 1.5-mL screw cap Eppendorf tube, vigorously mixed for 10 s, and placed in a heating cabinet at 97°C for 60 min. After cooling in a freezer at −20°C for 20 min, methanol (300 μL) and 20% TCA (100 μL) were added to the suspension and mixed for 10 s. The samples were centrifuged at 13,000×g for 6 min, and 100 μL of the supernatant was transferred to autosampler vials. As acid hydrolysis of TEP yields stoichiometric amounts of MDA, a standard curve was made from TEP dissolved in methanol and diluted in water at concentrations of 10.0, 5.0, 2.5, 1.25, 0.62, 0.31, 0.16 μM, and blank. TEP standards were heated at 50°C for 60 min and were stored in a refrigerator for maximum 1 week. Samples (20–40 serum samples per day) were thawed, derivatized, and measured. The standard curve generated with TEP standards from 0.0 to 10.0 μM was linear with a correlation coefficient \( r = 0.9991 \). The retention time of the TBA2–MDA adduct was 1.8 min at flow rate of 0.8 mL/min. Run time per sample were 4 min. The mean area of the small peak obtained with the blank control (H2O) was subtracted from the peak heights of calibration and samples.

**Plasma thiols assay**

**Reagents:**

- 10 mM DTNB (5-5 Dithio-bis(2- Nitrobenzoic Acid) in absolute methanol, 20 mg DTNB dissolved in 5 ml Methanol. Store at 0-4 degrees. Stable for up to 2 weeks. Use 15 ml graduated falcon conical centrifuge tube for storage.
- 0.25 M TRis Buffer with 20 mM EDTA at ph 8.2, dissolve 1.51375 g Tris (Trizma Base-MW 121.1) and 50 ml distilled water and adjust ph to 8.2 using 6N HCL. Use graduated conical centrifuge tubes for storage
- Absolute methanol

Protocol:

Completely thaw sample on ice before proceeding

50 ul plasma (50 ul Tris for sample blank)

| Add Tris Buffer 150 ul, vortex

| Add 10 ml DTNB (10 um MeOH for DTNB blank), vortex

| Add MeOH 790 ul, vortex

| Incubate 15 minutes at room temperature

| Centrifuge at 1000 rpm for 5 minutes

| Supernatant

| Take optical density at 412 nm against sample blank

Calculations:

(A-B) * 1470 = SH group in serum (uM)

A: optical density of sample
B: optical density of DTNB blank.
Appendix M: Sample size calculations for study 2

\[
N = \frac{\sigma_1^2 + \sigma_2^2}{\Delta^2} \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2
\]

\(\sigma_1\): is the standard deviation of the difference between post and pre plasma levels of the vitamins in \textit{H. pylori} positive group

\(\sigma_2\): is the standard deviation of the difference between post and pre plasma levels of the vitamins in \textit{H. pylori} negative group

\(\Delta\): is the effect size.

Assumptions:

1- \(\Delta\), the effect size is \(\frac{1}{2}\) standard deviation of cross sectional measure of plasma levels of vitamins (\(\sigma_{xs}\)).

2- \(\sigma_1 = \sigma_2 = \sigma\)

Calculation of \(\sigma\) in function of \(\sigma_{xs}\)

\[
Y = T_{post} - T_{pre}
\]

Where \(T_{post}\) is the post supplementation plasma levels and \(T_{pre}\) is the pre supplementation levels

\[
\text{Var}(Y) = \text{Var}(T_{post}) + \text{Var}(T_{pre}) - 2r \sqrt{\text{Var}(T_{post}) \times \text{Var}(T_{pre})}
\]

\[
= \sigma_{post}^2 + \sigma_{pre}^2 - 2r \sigma_{post} \sigma_{pre}
\]

\[
= 2 \sigma_{xs}^2 - 2r \sigma_{xs}^2
\]

\[
= 2 \sigma_{xs}^2 (1-r)
\]

“\(r\)” is the correlation between pre and post supplementation plasma levels of vitamin C and E in the same individuals.

If we assume that the correlation is 0.75 then

\[
\text{Var}(Y) = 0.5 \sigma_{xs}^2
\]

\[
\sigma = \sqrt{0.5 \sigma_{xs}}
\]

\[
N = \frac{\sigma_1^2 + \sigma_2^2}{\Delta^2} \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2
\]

\[
N = \frac{2 \sigma^2}{(0.5 \sigma_{xs})^2} \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2
\]

\[
N = \frac{2 \times 2 \sigma_{xs}^2 (1-r)}{(0.5 \sigma_{xs})^2} \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2
\]

\[
N = 16 (1-r) \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2
\]

If we consider \(r = 0.75\) then \(N = 32\)
## Appendix N: Summary of few supplementation studies for vitamins E and C

<table>
<thead>
<tr>
<th>Study</th>
<th>Dosage</th>
<th>Duration</th>
<th>Population (n)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Sanjo et al (1996)</td>
<td>Vit C: 250 mg</td>
<td>7 days</td>
<td>Patients with gastric lesions (43)</td>
<td>Plasma vit E &amp; C increased significantly</td>
</tr>
<tr>
<td></td>
<td>Vit E: 200 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gatto et al (1996)</td>
<td>Vit C: 1000 mg</td>
<td>4 weeks</td>
<td>Healthy females (10)</td>
<td>Plasma vit C levels peaked after 2 weeks</td>
</tr>
<tr>
<td>Sasaki et al (2000)</td>
<td>Vit C: 1000 mg</td>
<td>3 months</td>
<td>High risk for atrophic gastritis (55)</td>
<td>Plasma vit C peaked at one month</td>
</tr>
<tr>
<td>Erhardt et al (2000)</td>
<td>Vit E: 134.2 mg</td>
<td>7 days</td>
<td>Health females (12)</td>
<td>Plasma vit E increases significantly</td>
</tr>
<tr>
<td>Schneider et al (2001)</td>
<td>Vit C: 1000 mg</td>
<td>7 days</td>
<td>Smokers (12)</td>
<td>Plasma vit E &amp; C increases sig. in smokers</td>
</tr>
<tr>
<td></td>
<td>Vit E: 33505 mg</td>
<td></td>
<td>Non smokers (12)</td>
<td>not in non smokers</td>
</tr>
<tr>
<td>Sampson et al (2001)</td>
<td>Vit E: 400 IU</td>
<td>8 weeks</td>
<td>Health subjects (30)</td>
<td>Plasma vit E increased sig. in both groups</td>
</tr>
<tr>
<td></td>
<td>Diabetic (40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singhal et al (2001)</td>
<td>Vit C: 1000 IU</td>
<td>30 days</td>
<td>Healthy subjects (175)</td>
<td>Lipid peroxidation decreased sig. in both</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td></td>
<td></td>
<td>treatments</td>
</tr>
<tr>
<td></td>
<td>Vit E: 400 IU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vit E: 680 IU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Everett et al (2002)</td>
<td>Vit C: 1000 mg</td>
<td>4 weeks</td>
<td>Hpylori infected subjects (117)</td>
<td>Plasma Vit C &amp; E doubled</td>
</tr>
<tr>
<td></td>
<td>Vit E: 100 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bader et al (2006)</td>
<td>Vit C: 500 mg</td>
<td>4 weeks</td>
<td>Health men (19)</td>
<td>Plasma vit C: 28% increase</td>
</tr>
<tr>
<td></td>
<td>Vit E: 272 IU</td>
<td></td>
<td></td>
<td>Plasma vit E: 32 % increase</td>
</tr>
<tr>
<td>Dietrich et al (2002)</td>
<td>Vit C: 515± 28 g</td>
<td>60 days</td>
<td>Smokers (42)</td>
<td>Plasma vit C: 69.9% increase</td>
</tr>
<tr>
<td>Dietrich et al (2002)</td>
<td>Vit C: 515± 28 mg</td>
<td>60 days</td>
<td>Smokers (39)</td>
<td>Plasma vit C: 89.7%</td>
</tr>
<tr>
<td></td>
<td>Vit E: 371±56 mg</td>
<td></td>
<td></td>
<td>Plasma vit E: 33.8%</td>
</tr>
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</table>


## Appendix O: Results of the weighted analysis of study 1

Frequency distribution, % seropositivity, odds ratio estimates and their 95% confidence intervals in males and females by age.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) † (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) † (95% CI)</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) † (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) † (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>&lt;60 yrs</td>
<td>218</td>
<td>29.4 (23.7-35.8)</td>
<td>27.7 (17.6-41.0)</td>
<td>1</td>
<td>1</td>
<td>191</td>
<td>17.3 (12.5-23.3)</td>
<td>10.5 (5.9-18.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60-70 yrs</td>
<td>321</td>
<td>32.1 (27.2-37.4)</td>
<td>31.96 (27.0-37.4)</td>
<td>1.1</td>
<td>1.2</td>
<td>243</td>
<td>24.3 (19.3-30.1)</td>
<td>1.5 (1.0-2.5)</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>&gt;70 yrs</td>
<td>194</td>
<td>38.7 (32.0-45.7)</td>
<td>38.7 (32.0-45.8)</td>
<td>1.5</td>
<td>1.6</td>
<td>139</td>
<td>36.0 (28.4-44.3)</td>
<td>2.7 (1.6-4.5)</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>733</td>
<td>33.0 (29.7-36.5)</td>
<td>29.4 (21.1-39.3)</td>
<td>1</td>
<td>1</td>
<td>573</td>
<td>24.8 (21.4-28.5)</td>
<td>14.9 (10.1-21.4)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

† Weighted values presented

Frequency distribution, % seropositivity, age adjusted odds ratio estimates and their 95% confidence intervals in males and females.

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) † (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) † (95% CI)</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) † (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) † (95% CI)</th>
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<tbody>
<tr>
<td>Single</td>
<td>90</td>
<td>40.0 (30.4-50.4)</td>
<td>40.8 (20.3-65.1)</td>
<td>1</td>
<td>1</td>
<td>194</td>
<td>27.8 (22.0-34.6)</td>
<td>20.8 (13.1-21.3)</td>
<td>1</td>
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<tr>
<td>Married</td>
<td>633</td>
<td>32.1 (28.5-35.8)</td>
<td>30.2 (21.4-40.8)</td>
<td>0.7</td>
<td>0.6</td>
<td>374</td>
<td>23.3 (19.2-27.8)</td>
<td>12.8 (7.7-20.6)</td>
<td>0.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

† Weighted values presented
<table>
<thead>
<tr>
<th>Place of birth</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
<th>Females no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
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<tbody>
<tr>
<td>Canada</td>
<td>462</td>
<td>26.6</td>
<td>(22.8-30.8)</td>
<td>1</td>
<td>1</td>
<td>396</td>
<td>25.3</td>
<td>(21.2-29.8)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Other</td>
<td>271</td>
<td>43.9</td>
<td>(38.1-49.9)</td>
<td>2.2</td>
<td>1.6</td>
<td>177</td>
<td>23.7</td>
<td>(18.0-30.6)</td>
<td>0.8</td>
<td>1.1</td>
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<tr>
<td>Ethnicity</td>
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<tr>
<td>White</td>
<td>676</td>
<td>32.0</td>
<td>(28.5-35.56)</td>
<td>1</td>
<td>1</td>
<td>534</td>
<td>24.7</td>
<td>(21.2-28.6)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Non white</td>
<td>56</td>
<td>46.4</td>
<td>(33.9-59.5)</td>
<td>1.8</td>
<td>0.7</td>
<td>38</td>
<td>23.7</td>
<td>(12.8-39.6)</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Age of immigration</td>
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<tr>
<td>&lt;20 yrs</td>
<td>111</td>
<td>34.2</td>
<td>(26-43.5)</td>
<td>1</td>
<td>1</td>
<td>62</td>
<td>21.1</td>
<td>(12.6-32.9)</td>
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<td>1.0</td>
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<tr>
<td>&gt;20 yrs</td>
<td>152</td>
<td>51.3</td>
<td>(43.4-59.2)</td>
<td>1.9</td>
<td>1.5</td>
<td>111</td>
<td>26.1</td>
<td>(16.9-42.5)</td>
<td>1.2</td>
<td>2.5</td>
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<td>Education</td>
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<tr>
<td>Low</td>
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<td>35.2</td>
<td>(29.8-41.0)</td>
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<td>1</td>
<td>225</td>
<td>29.3</td>
<td>(23.7-35.6)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Middle</td>
<td>232</td>
<td>37.9</td>
<td>(31.9-44.4)</td>
<td>1.1</td>
<td>1.0</td>
<td>215</td>
<td>25.6</td>
<td>(20.1-31.8)</td>
<td>0.9</td>
<td>0.9</td>
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<tr>
<td>High</td>
<td>212</td>
<td>25.0</td>
<td>(19.6-31.3)</td>
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<td>0.5</td>
<td>123</td>
<td>15.4</td>
<td>(10-23)</td>
<td>0.5</td>
<td>0.4</td>
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<tr>
<td>Income</td>
<td>Total no.</td>
<td>Total %+ve (95% CI)</td>
<td>Males %+ve (W) (95% CI)</td>
<td>OR (95% CI)</td>
<td>Males OR (W) (95% CI)</td>
<td>Total no.</td>
<td>%+ve (95% CI)</td>
<td>%+ve (W) (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (W) (95% CI)</td>
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<tr>
<td>--------</td>
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<tr>
<td>&lt;20 K</td>
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<td>42.2 (35.2-49.4)</td>
<td>44.4 (27.3-63.0)</td>
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<td>1</td>
<td>207</td>
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<td>12.8 (5.2-28.0)</td>
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<td>20-40K</td>
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<td>41.2 (26.4-57.8)</td>
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<td>0.9</td>
<td>159</td>
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<tr>
<td>&gt;40K</td>
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<td>26.3 (11.4-49.8)</td>
<td>21.7 (8.5-45.3)</td>
<td>0.5</td>
<td>0.3</td>
<td>24</td>
<td>25.0 (11.7-45.7)</td>
<td>22.4 (9.7-43.6)</td>
<td>0.9</td>
<td>1.2</td>
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<tr>
<td>Missing</td>
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<td>18.7 (10.6-30.7)</td>
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<td>0.3</td>
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<table>
<thead>
<tr>
<th>Number of siblings</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
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<tr>
<td>&lt;2</td>
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<td>25.0 (13.7-41.0)</td>
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<td>10.7(5.6-19.6)</td>
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<tr>
<td>2-4</td>
<td>180</td>
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<td>31.2 (18.5-47.9)</td>
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<td>1.4</td>
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<td>27.3 (20.1-35.4)</td>
<td>16.9 (10.1-26.8)</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>&gt;4</td>
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<td>38.6 (31.3-46.4)</td>
<td>48.1 (30.7-66.0)</td>
<td>1.5</td>
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<td>116</td>
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<td>Missing</td>
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<td>16.3 (8.2-29.8)</td>
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<td>0.6</td>
<td>63</td>
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</table>

<table>
<thead>
<tr>
<th>Regular use of antacids</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
<th>Total no.</th>
<th>%+ve (95% CI)</th>
<th>%+ve (W) (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (W) (95% CI)</th>
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<tbody>
<tr>
<td>Yes</td>
<td>100</td>
<td>44.0 (34.6-53.9)</td>
<td>64.7 (43.5-81.4)</td>
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<td>1</td>
<td>88</td>
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<tr>
<td>No</td>
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<th>OR (W) (95% CI)</th>
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<th>OR (95% CI)</th>
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<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29.6-37.1)</td>
<td>(21.9-41.4)</td>
<td></td>
<td>(0.6-1.8)</td>
<td></td>
<td>(21.3-29)</td>
<td>(9.8-22.4)</td>
<td></td>
<td>(0.6-1.9)</td>
</tr>
</tbody>
</table>

†Weighted values presented
**Final weighted multivariate model**

<table>
<thead>
<tr>
<th></th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>1</td>
</tr>
<tr>
<td>60-70</td>
<td>1.2 (0.7-2)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1.6 (0.9-2.8)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>0.3 (0.1-0.6)</td>
</tr>
<tr>
<td><strong>Number of Siblings</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>2-4</td>
<td>1.4 (0.8-3.1)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>3.0 (1.4-6.5)</td>
</tr>
<tr>
<td><strong>Alcohol Intake</strong></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
</tr>
<tr>
<td>&lt;10d/week</td>
<td>0.5 (0.3-1)</td>
</tr>
<tr>
<td>&gt;10d/week</td>
<td>0.4 (0.2-0.8)</td>
</tr>
<tr>
<td><strong>Interaction Sex*age</strong></td>
<td></td>
</tr>
<tr>
<td>Sex(Females)*age(60-70)</td>
<td>1.9 (0.8-4.8)</td>
</tr>
<tr>
<td>Sex(Females)*age(&gt;70)</td>
<td>3.1 (1.2-8.1)</td>
</tr>
</tbody>
</table>
Appendix P: Dietary intakes of *H. pylori* negative and positive subjects (unadjusted for energy) (study 2)

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>p-value*</th>
<th>Week 4</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. pylori –ve</strong></td>
<td></td>
<td></td>
<td><strong>H. pylori +ve</strong></td>
<td></td>
</tr>
<tr>
<td>n=32</td>
<td></td>
<td></td>
<td>n=26</td>
<td></td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>2164.87± 500.06</td>
<td>0.10</td>
<td>2043±441.88</td>
<td>0.24</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>278.14 ±72.88</td>
<td>0.02</td>
<td>272.00±64.62</td>
<td>0.57</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>86.45 ±23.26</td>
<td>0.07</td>
<td>80.95±19.77</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>79.60± 27.11</td>
<td>0.16</td>
<td>70.82±20.56</td>
<td>0.52</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>21.40 ±8.35</td>
<td>0.13</td>
<td>19.85±7.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>1.55± 0.52</td>
<td>0.22</td>
<td>1.46±0.48</td>
<td>0.57</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.66 ±0.41</td>
<td>0.14</td>
<td>1.63±0.51</td>
<td>0.79</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>20.81 ± 6.71</td>
<td>0.16</td>
<td>19.40±6.90</td>
<td>0.47</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>1.77± 0.82</td>
<td>0.03</td>
<td>1.52±0.62</td>
<td>0.11</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>3.73± 2.51</td>
<td>0.98</td>
<td>3.97±2.10</td>
<td>0.24</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>395.53± 265.73</td>
<td>0.85</td>
<td>391.53±207.19</td>
<td>0.94</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>112.31 ±59.17</td>
<td>0.06</td>
<td>111.83±65.04</td>
<td>0.21</td>
</tr>
<tr>
<td>Vitamin D (mg)</td>
<td>2.61 ±1.82</td>
<td>0.13</td>
<td>2.54±1.88</td>
<td>0.71</td>
</tr>
<tr>
<td>Vitamin A (µg)</td>
<td>1936.60±994.50</td>
<td>0.50</td>
<td>2204.42±922.48</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>5.20 ±2.27</td>
<td>0.08</td>
<td>4.69±1.71</td>
<td>0.20</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>793.16 ±230.65</td>
<td>0.01</td>
<td>762.64±281.42</td>
<td>0.12</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>16.84 ±5.56</td>
<td>0.03</td>
<td>16.19±7.00</td>
<td>0.27</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>260.57 ±91.72</td>
<td>0.02</td>
<td>234.52±77.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2484.12±691.68</td>
<td>0.06</td>
<td>2372.90±761.56</td>
<td>0.23</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3207.37± 856.05</td>
<td>0.02</td>
<td>3229.96±1108.10</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Appendix Q: Results for plasma alpha tocopherol after adjustment for lipid expressed as (mg/g lipid) (study 2).

The results of the adjusted (mg/g lipids) and unadjusted (umol/l) plasma alpha tocopherol were comparable. Post supplementation levels of alpha tocopherol were significantly higher than pre supplementation levels in *H. pylori* (−3.74±2.98 vs. 2.29±1.37; p<0.05); In *H. pylori* +ve, although post supplementation levels were slightly higher than pre supplementation, this difference did not reach statistical significance (3.57±2.44 vs. 2.77±3.09; ns). *H. pylori* –ve and +ve groups were comparable with regard to pre and post supplementation levels, change and proportional in lipid adjusted plasma alpha tocopherol levels.

Table Pre and post supplementation plasma alpha tocopherol adjusted for lipids (mg/g lipids) in *H. pylori* –ve and +ve subjects

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> –ve (n=32)</th>
<th><em>H. pylori</em> +ve (n=26)</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre supplementation</td>
<td>2.36±1.33</td>
<td>2.85±3.12</td>
<td>0.42</td>
</tr>
<tr>
<td>Post supplementation</td>
<td>3.70±2.98*</td>
<td>3.25±1.85</td>
<td>0.51</td>
</tr>
<tr>
<td>p-value*</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Change (post-pre)</td>
<td>1.33±2.77</td>
<td>0.40±3.41</td>
<td>0.25</td>
</tr>
<tr>
<td>Proportional change (% change)</td>
<td>84.11±128.48</td>
<td>60.28±99.88</td>
<td>0.42</td>
</tr>
</tbody>
</table>

† Values in this table represent the means ± SD
* p-value derived from independent t test for comparison of means between *H. pylori* negative and positive group (log transformed data)
** p-value derived from paired t test for comparison of pre and post supplementation plasma levels (log transformed data)
Appendix R: Association of sex and plasma ascorbic acid, alpha tocopherol, malondialdehyde and thiols (study 2).

Plasma concentrations of ascorbic acid, alpha tocopherol, malondialdehyde and thiols in males and females†.

<table>
<thead>
<tr>
<th></th>
<th>Males (n=24)</th>
<th>Females (n=33)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascorbic acid (umol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre supplementation</td>
<td>45.28±15.84</td>
<td>54.14±21.42</td>
<td>0.18</td>
</tr>
<tr>
<td>Post supplementation</td>
<td>64.04±23.64</td>
<td>69.64±16.70</td>
<td>0.17</td>
</tr>
<tr>
<td>Change</td>
<td>18.76±25.14</td>
<td>15.50±19.90</td>
<td>0.58</td>
</tr>
<tr>
<td>Proportional Change (%)</td>
<td>56.61±70.86</td>
<td>51.80±85.97</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Alpha tocopherol (umol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre supplementation</td>
<td>23.44±6.83</td>
<td>22.11±5.93</td>
<td>0.46</td>
</tr>
<tr>
<td>Post supplementation</td>
<td>40.46±18.38</td>
<td>35.60±8.87</td>
<td>0.76</td>
</tr>
<tr>
<td>Change</td>
<td>17.02±16.00</td>
<td>13.79±8.78</td>
<td>0.96</td>
</tr>
<tr>
<td>Proportional Change (%)</td>
<td>76.50±61.51</td>
<td>69.08±48.76</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Malondialdehyde (umol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre supplementation</td>
<td>0.013±0.014</td>
<td>0.013±0.010</td>
<td>0.71</td>
</tr>
<tr>
<td>Post supplementation</td>
<td>0.009±0.004</td>
<td>0.009±0.006</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Thiols (umol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre supplementation</td>
<td>543.73±209.78</td>
<td>500.72±119.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Post supplementation</td>
<td>491.41±172.73</td>
<td>500.76±177.03</td>
<td>0.74</td>
</tr>
</tbody>
</table>

† Values in this table represent means ± SD
* p-value derived from independent t test of means of log transformed data