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DIFFERENCES IN KIMCHI AND GLUTATHIONE INTAKE IN KOREANS VS. KOREAN-AMERICANS: POSSIBLE ROLE IN PATHOGENESIS OF STOMACH CANCER

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
Kyeok Kim, M.S.

The Ohio State University
1997

Dissertation Committee:
Professor Wayne Johnson, Adviser
Professor Tammy M Bray
Professor Alma Saddam

Approved by

The Ohio State University Nutrition Program
ABSTRACT

Gastric cancer kills over a half million people worldwide each year. The 25-fold variation in incidences among different countries supports the concept that environmental factors, particularly food components, are major factors involved in the disease. Korea has the highest stomach cancer rate in the world. However, the rate of gastric cancer in Korean-Americans is very low. This suggested that a unique food, which is consumed frequently and consistently in Korea, might be responsible for the high incidence of stomach cancer. An epidemiological study was performed to compare the dietary patterns between Koreans from Seoul and its vicinity and Korean Americans from Columbus, Ohio. In addition, selected antioxidant nutrient intakes were compared between these two groups. Three hundred questionnaires for each group, including personal health and two-day dietary records, were distributed to adults more than 35-years old in Seoul, Korea and in Columbus, Ohio. It was found that Kimchi, a fermented spicy Chinese cabbage and a traditional staple food in Korea, was consumed in significantly higher amounts by Koreans (>60g/day) compared to Korean-Americans (<20g/day). In addition, daily antioxidant intakes of vitamin C, E
and glutathione in Korean-Americans were significantly higher than those in Koreans. The nutrient content of the foods was calculated from the data provided by the USDA Food Composition Database. However, glutathione, one of the important antioxidant components in food which may play a critical role in protecting the stomach at the mucosal site, was not available from the database for calculation. Measurement of the glutathione content of Korean foods selected from the questionnaire was performed. The results demonstrated that the Korean-Americans' favorite Korean foods such as seaweed or brown rice had more glutathione than favorite foods of Koreans such as white rice, red pepper paste, soybean paste stew, and Kimchi. From the epidemiology study, it was hypothesized that Kimchi, fermented Chinese cabbage with salt, hot pepper, and other minor ingredients, promotes gastric carcinogenesis by decreasing the gastric antioxidant and detoxification enzymes due to spice, the high salt concentration, and the fermentation process. These enzyme systems constitute the first line of defense in protection against damage caused by free radicals and carcinogens. Thus, the objective of this study was to determine the effect of dietary Kimchi on the glutathione contents, and on the activities of antioxidant enzymes, glutathione peroxidase, glutathione reductase and glutathione s-transferase in liver, and digestive organs such as the stomach, small intestine, and colon of Wistar rats. Male Wistar rats were fed four different diets including 5 % Cellulose, 5 % Kimchi, 10 % Cellulose, and 10 % Kimchi in 76 AIN modified diet for 2 weeks and 4 weeks. The control group rats were fed basal diet for 5 days and sacrificed. Results showed that a high
intake of Kimchi decreased antioxidant enzyme activities and weakened the first line of defense against carcinogens in the stomach. Results showed indicated that the 2-week diets had significant differences among different diet groups but the 4-week diets did not show significant differences.

Glutathione peroxidase activity in the stomach showed significant difference between cellulose and Kimchi groups in 2 weeks. In 4 weeks, significant difference was found between 5% cellulose and 10% Kimchi groups. Even though no significant differences were found in glutathione reductase and glutathione S-transferase activities among treatment groups, the activities were the lowest in the 10% Kimchi groups, suggesting that high Kimchi intake can impair antioxidant enzyme activity in the stomach.

The current research provided the understanding of dietary factors that could influence the susceptibility to cancer and the knowledge to design preventive measures, by studying the diet and disease patterns between Koreans and Korean-Americans.
Dedicated to my parents
ACKNOWLEDGMENTS

I thank Dr. Tammy M. Bray for giving me a chance to explore the world of antioxidants, and Dr. Wayne Johnson and Dr. Alma Saddam for their timely and appropriate advice. I also want to thank everyone in the nutrition laboratory. I thank my parents in Korea for their unending support at all times.
VITA

May 24, 1960 ......................... Born in Seoul, Korea

Feb. 1992 ................................. M.S. in Food and Nutrition at DukSung
Women's University, Seoul, Korea


1996-present ............................ Graduate Teaching and Research Associate,
The Ohio State University

PUBLICATIONS

Food Sci. 7:21-27.

Dietary Patterns and Antioxidant intake between Korean-Americans and
Koreans, and Their Possible Causative Relationship with Stomach Cancer.
FASEB J.Abstract;3393

Glutathione Contents of Selected Korean Foods and Comparison of Dietary
Abstract: PT 383
ACHIEVEMENTS

1996. November: Graduate Student Alumni Research Award

1997. February: College of Human Ecology Graduate Student Travel Awards

1997. April: 1st place poster Presentation, Graduate Research Forum  
(College of Human Ecology)

1997. May: Virginia M. Vivian Award

FIELD OF STUDY

Major Field: Ohio State University Nutrition Program

Specialty: Nutritional Toxicology
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>v</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>vi</td>
</tr>
<tr>
<td>Vita</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiv</td>
</tr>
<tr>
<td>CHAPTER 1 - LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>1.1. Type of adenocarcinoma</td>
<td>5</td>
</tr>
<tr>
<td>1.2. Mechanism of stomach cancer</td>
<td>6</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>9</td>
</tr>
<tr>
<td>1.3. Antioxidant Enzymes in the Cellular Defense Systems</td>
<td>12</td>
</tr>
<tr>
<td><em>N-nitroso compound</em></td>
<td>13</td>
</tr>
<tr>
<td>1.4. Dietary factors influencing risk of gastric cancer</td>
<td>15</td>
</tr>
<tr>
<td><em>Fresh vegetables and fruits</em></td>
<td>15</td>
</tr>
<tr>
<td><em>Vitamins</em></td>
<td>16</td>
</tr>
<tr>
<td><em>Glutathione (GSH)</em></td>
<td>18</td>
</tr>
<tr>
<td><em>Function of Glutathione</em></td>
<td>19</td>
</tr>
<tr>
<td><em>Glutathione peroxidase</em></td>
<td>23</td>
</tr>
<tr>
<td><em>Glutathione Reductase</em></td>
<td>24</td>
</tr>
<tr>
<td><em>Glutathione S-Transferase</em></td>
<td>25</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1.1: Typical ingredients of Kimchi ................................................................. 30
Table 3.1: Demographic characteristics of study subjects .................................... 43
Table 3.2: Characteristics of study subjects in Columbus, OH and Seoul, Korea .......... 45
Table 3.3: Comparison of daily nutrients ingested by Korean-American male and Korean female subjects ................................................................. 47
Table 3.4: Comparison of daily nutrients ingested by Korean-American female and Korean female subjects ................................................................. 57
Table 3.5: Characteristics of Korean-Americans ......................................................... 61
Table 4.1: Glutathione content in selected Korean foods .......................................... 74
Table 5.1: Comparison of rat diets (g/100 g) ............................................................. 82
Table 5.2: Effects of dietary Kimchi intake on feed intake and weight gain of rats (2 weeks) ................................................................. 86
Table 5.3: Effect of dietary Kimchi intake on feed intake and weight gain of rats (4 weeks) ................................................................. 87
Table 5.4: GSH contents in the rat liver ................................................................. 93
Table 5.5: GSH contents in the rat stomach .............................................................. 95
Table 5.6: GSH contents in the rat small intestine ......................................................... 97
Table 5.7: GSH contents in the rat colon ................................................................. 99
Table 5.8: GPx activity in the rat liver ................................................................. 101
Table 5.9: GPx activity in the rat stomach.................................................................103
Table 5.10: GPx activity in the rat small intestine. .................................................105
Table 5.11: GPx activity in the rat colon.................................................................107
Table 5.12: Glutathione reductase activity in the rat liver ....................................109
Table 5.13: Glutathione reductase in the rat stomach............................................111
Table 5.14: Glutathione reductase in the rat small intestine.................................113
Table 5.15: Glutathione reductase activity in rat colon........................................115
Table 5.16: GST activity in the rat liver.................................................................117
Table 5.17: GST activity in the rat stomach............................................................119
Table 5.18: GST activity in the rat small intestine.................................................121
Table 5.19: GST activity in the rat colon...............................................................123
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1: Age-adjusted male stomach cancer mortality per 100,000 population (adapted from Boring (12))</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.2: Mechanism of stomach cancer (adapted from Kozol (18))</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.3: <em>Helicobacter pylori</em> beneath the mucosa layer of the stomach (18)</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.4: The role of free radicals in the pathogenesis of stomach cancer</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.5: The role of antioxidants in the foods in the pathogenesis of stomach cancer</td>
<td>17</td>
</tr>
<tr>
<td>Figure 1.6: Prevention of stomach cancer</td>
<td>27</td>
</tr>
<tr>
<td>Figure 1.7: The role of Kimchi in the function of antioxidant enzymes in the mucous membrane</td>
<td>31</td>
</tr>
<tr>
<td>Figure 3.1: The comparison of glutathione intake between Korean-American male and Korean male subjects</td>
<td>49</td>
</tr>
<tr>
<td>Figure 3.2: The comparison of zinc intake between Korean-American male and Korean male subjects</td>
<td>50</td>
</tr>
<tr>
<td>Figure 3.3: The comparison of iron intake between Korean-American male and Korean male subjects</td>
<td>51</td>
</tr>
<tr>
<td>Figure 3.4: The comparison of copper intake between Korean-American male and Korean male subjects</td>
<td>52</td>
</tr>
<tr>
<td>Figure 3.5: The comparison of Animal protein intake between Korean-American male and Korean male subjects</td>
<td>53</td>
</tr>
<tr>
<td>Figure 3.6: The comparison of saturated fatty acid intake between Korean-American male and Korean male subjects</td>
<td>54</td>
</tr>
</tbody>
</table>
Figure 3.7: The comparison of monounsaturated fatty acid intake between Korean-American and Korean male subjects ................................................................. 55

Figure 3.8: The comparison of polyunsaturated fatty acid intake between Korean-American and Korean male subjects ........................................................................... 56

Figure 3.9: Intake of major dietary factors between Koreans and Korean-Americans ......................................................................................................................................... 59

Figure 5.1: Accumulative food intake in different diet rats (2weeks) ........................................ 88

Figure 5.2: Accumulative weight gain in different diet rats (4weeks) .................................. 89

Figure 5.3: The relationship between food intake and weight gain for four treatment groups ........................................................................................................................................ 90

Figure 5.4: The relationship between food intake and weight gain for 5% cellulose and 5% Kimchi groups .................................................................................................. 91

Figure 5.5: The relationship between food intake and weight gain for 10% cellulose and 10% Kimchi groups ........................................................................................................ 92

Figure 5.6: Glutathione contents in the liver ........................................................................ 94

Figure 5.7: Glutathione contents in the stomach ................................................................ 96

Figure 5.8: Glutathione content in the small intestine .......................................................... 98

Figure 5.9: Glutathione contents in the colon ......................................................................... 100

Figure 5.10: Glutathione peroxidase activity in the liver .................................................... 102

Figure 5.11: Glutathione peroxidase activity in the stomach ............................................... 104

Figure 5.12: Glutathione peroxidase activity in the small intestine ...................................... 106

Figure 5.13: Glutathione peroxidase activity in the colon .................................................. 108

Figure 5.14: Glutathione reductase activity in the liver ....................................................... 110

Figure 5.15: Glutathione reductase activity in the stomach ................................................ 112

Figure 5.16: Glutathione reductase activity in the small intestine ....................................... 114
Figure 5.17: Glutathione reductase activity in the colon ......................................... 116
Figure 5.18: Glutathione S-transferase activity in the liver ...................................... 118
Figure 5.19: Glutathione S-transferase activity in the stomach ............................... 120
Figure 5.20: Glutathione S-transferase activity in the small intestine ..................... 122
Figure 5.21: Glutathione S-transferase activity in the colon .................................... 124
CHAPTER 1

LITERATURE REVIEW

Epidemiology is defined as the study of the distribution and determinants of disease frequency in human populations and has two fundamental assumptions: first, human disease does not occur at random; and second, human disease has causal and preventive factors that can be identified through systematic investigation of different populations or subgroups of individuals within a population in different places or at different times (1).

Epidemiological studies regarding to a stomach cancer were performed even before the Second World War (2). During the 1950s and 1960s, epidemiological research of stomach cancer was very active. By the end of the 1970s, almost 20 case-control studies had been reported (3). In addition, cohort studies had been used in the 1960s to determine the risk factors of a stomach cancer.

It was estimated that stomach cancer was the worldwide leading cause of cancer incidence among all cancer sites at the beginning of the 1980s with 670,000 new cases each year (4). However, stomach cancer incidence and mortality in the highly industrialized countries declined remarkably during the last decades (5).
Nonetheless stomach cancer was the leading cause of cancer in the United States at the beginning of this century, it is now the 10th and 12th most common cancer incidence with a decline in mortality of about 70% since the 1950s (6). Other countries are following the trend in the US but with some delay (7). Thus, worldwide many countries are still confronted with high incidences of this disease.

Overall, the results from these early epidemiological studies do not show a consistent pattern of factors associated with the disease, although, individually for each study, many associations were identified. Decreased intake of milk, fruit, and vegetables, increased fried food and alcohol consumption, and more tobacco smoking were most often found to be associated with risk of stomach cancer. Whereas epidemiological research could not present consistent results regarding risk factors for stomach cancer at the end of the 1970s, major progress was made in experimental research, which was detection of N-nitroso compounds. It was found that N-nitroso compounds could induce glandular stomach cancer in animals. The N-nitroso compound hypothesis, in most parts established by experimental research in the 1970s, directed the epidemiological research into risk factors of stomach cancer in the 1980s. Most interest concentrated on precursor substances, such as nitrate and nitrite, and substances that were able to inhibit the formation of N-nitroso compounds such as vitamin C (8).

Epidemiological study on stomach cancer showed that there were regional variations in the incidence of stomach cancer. In China, higher incidence of stomach
cancer was reported in the middle region and even higher incidence in the northern region. Regional variation in stomach cancer mortality was also found in western Europe. Areas in northern Portugal, in Central Spain, northern Italy and southeast Germany could be considered as high-risk regions for stomach cancer in western Europe, while Denmark, Greece, southern France and England could be considered as low-risk areas. Even in the same country the regional variation was conspicuous; there was 2.5-fold difference in stomach cancer incidence between low-risk area in the highly urbanized states and high-risk area (9).

Stomach cancer is prevalent all over the world and inter-country and intra-country differences in incidence exist, as well as different mortality rates between first and second generation migrants. The stomach is where food first comes into prolonged contact with the gastrointestinal mucosa and the human diet contains a variety of both carcinogens and carcinogen antagonists. From these findings, it is reasonable to assume that environmental factors, particularly dietary factors, have the most important etiologic implications for stomach cancer (5).

The precise components of diet which may have some impact on gastric carcinogenesis are, however, still largely undefined. Most studies based on food items found protective effects of fresh fruits and vegetables, while preserved meat and selected starchy foods—often indicators of a less affluent diet—were associated with increased risk (10). Scanty epidemiological data are, however, available on the role of nutrients, and micronutrients, on gastric cancer risk. There are indications that
selected micronutrients with antioxidant effect, such as ascorbic acid, beta carotene, and alpha-tocopherol, are protective against gastric carcinogenesis, while nitrites, while may cause intragastric synthesis of N-nitroso compounds, have been associated with increased risk, but their impact on a population level remains open for discussion. Diet has long been thought to be involved in the etiology or pathogenesis of gastric cancer, but the causes have proven elusive. The major diet-related factors implicated in increasing risk of the disease include high concentrations of nitrates/nitrites, present in some vegetables and in smoked, pickled, fermented, salted foods; extremely high salt intakes and factors fostering nitrosamine formation. The most popular hypothesis is that salt acts as an irritant to the gastric mucosa and intra-gastric nitrosamide formation from exogeneous nitrate and nitrite have a carcinogenic effect, modulated by other protective factors such as vitamin C (11).

When the stomach cancer mortality was compared world wide, Korea was the leading country with 57.9 deaths per 100,000 for males and 26.1 deaths per 100,000 for females over the period of 1986-1987 (Figure 1.1) (12). Since 1990, the results of 3 case-control studies (13-15) and one cohort study (16) on stomach cancer have been reported in Korea. Several food items were associated with increased or decreased risk of stomach cancer among Koreans. An increased risk of stomach cancer was noted among individuals who frequently consume broiled fishes and meats, soybean paste thick stew and hot pepper soybean paste stew. Frequent intake of green
vegetables such as spinach, cabbage, and soybean foods such as tofu decreased the risk. Consumption of pickled vegetables increased risk, while fresh vegetables did not.

Figure 1.1: Age-adjusted male stomach cancer mortality per 100,000 population (adapted from Boring (12))

1.1. Type of adenocarcinoma

Most gastric cancers are adenocarcinomas of either the intestinal type, characterized by cohesive neoplastic cells that form glandlike tubular structures and a
discrete mass, or of the diffuse type, in which cancerous cells diffusely infiltrate the stomach wall. Intestinal type carcinomas often are accompanied by chronic gastritis and, especially, intestinal metaplasias that are thought to be precursors of dysplastic changes and of intestinal type cancers. Pathogenic differences between the two types of adenocarcinoma are also reflected in the observation that patients with diffuse-type carcinogen often belong to blood group A, indicating a possible influence of genetic factors for cancer risk, whereas environmental factors, e.g. *Helicobacter pylori* infection are possibly important for the development of intestinal-type cancers (17).

1.2. Mechanism of stomach cancer

Figure 1.2 shows the possible sequence of events for stomach cancer. The first step in development of gastric cancer involves chronic superficial gastritis, a condition that is completely reversible if the causative factors can be identified and removed. The next stage in progression is the development of chronic atrophic gastritis, with loss of parietal cell mass, eventually resulting in hypochlorydria. This allows bacterial overgrowth in the stomach. There seems to be a consensus that the first events, which can be linked to the precancerous process, are the chronic inflammatory changes in the gastric mucosa. Chronic gastritis, which can lead to permanent gland loss (i.e. atrophy), has been etiologically linked to gastric cancer. In chronic atrophic gastritis epithelial cells and glands are lost repeatedly over a span of many years. Cells with intestinal phenotype replace them. In this process the adult gastric epithelial cells are replaced by another adult cells, metaplastic intestinal cells. This transformation from
gastric to intestinal cells seems to be gradual, as shown by the existence of cells, which display gastric and intestinal types of mucin. Gastric precancerous process involves a gradual loss of cell differentiation. The final product is a neoplastic cell that expresses phenotypes of different gastric and intestinal cells of multipotential cells found in the glandular necks. Irritants such as sodium chloride induce atrophy and facilitate the action of mutagens in the induction of dysplasia. Excessive salt is associated with gastric cancer risk by causing mucosal damage, and inflammatory changes. Also, salt promotes intragastric nitrosamine formation, causing excessive cell replication, and thereby perhaps potentiating the action of carcinogens and increasing endogenous mutation rates.
Normal Mucosa

Superficial Gastritis

Atrophic Gastritis

Dysplasia

Carcinoma

Metastasis

Figure 1.2: Mechanism of stomach cancer (adapted from Kozol (18))
Research on the effects of the environmental factors on gastric cancer has mainly focused on diet. Recently, the potential role of Helicobacter pylori, a gram-negative, spiral bacteria in stomach cancer pathogenesis has been actively studied, given that H. pylori has been commonly found in patients with severe gastritis and chronic atrophic gastritis (19). Figure 1.3 shows the location of H. pylori.

H. pylori infection is extraordinarily common, especially in some developing countries, most adults are infected with H. pylori infection begins in childhood and continues throughout life (20). It was predicted that stomach cancer risk in a population with 100 % H. pylori infection would be higher by a factor of six than in a population without the infection (21). Also, the patients with a stomach cancer were found to have H. pylori infection twice more often than healthy controls (19).

However, since a stomach cancer develops only in a relatively small portion of the infected people with H. pylori, H. pylori itself may not be sufficient to produce gastric cancer without other risk factors. The various factors (e.g., diet) that may influence the process must be further studied along with H. pylori infection (21).
Figure 1.3: *Helicobacter pylori* beneath the mucosa layer of the stomach (18)
The exact mechanisms through which *H. pylori* infection produces gastric
cancer remains unanswered. One possible mechanism is a nonspecific superficial
 gastritis due to *H. pylori* infection. In the presence of *H. pylori* along with other
factors, including dietary factors, superficial gastritis may progress to atrophic
gastritis, and eventually to the intestinal type of adenocarcinoma (19). *H. pylori*
infection causes local immune response from body. Inflammatory reaction produces
free radicals. Then, a high amount of free radicals may produce the stomach cancer.
*H. pylori* produces a factor which temporarily down-regulates acid secretion by
parietal cells. Therefore, under these conditions the stomach has higher pH than
normal. Bacteria can be overgrown in that situation. Another potential mechanism is
that *H. pylori* alters the metabolism of nitrites, and the nitrosamines or other N-nitroso
compounds cause the gastric cancer (20). Other factors such as increased gastric pH,
low levels of pepsin, altered mucus and the presence of increased numbers of bacteria
play a role in altered metabolism of nitrogenous substances in the presence of *H.
pylori*. Persons infected with *H. pylori* have lower levels of ascorbic acid in their
gastric juice, further enhancing these conversions (22). Therefore, *H. pylori* increases
the likelihood of atrophic gastritis by providing a milieu that is low in ascorbic acid
and high in nitroso compounds, thus setting the stage for enhanced risk (23).
1.3. Antioxidant Enzymes in the Cellular Defense Systems

Oxygen free radicals (OFR) are continuously generated in cells exposed to an aerobic environment. Antioxidant defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from OFR. Despite the antioxidant defense, OFR-related damage of proteins and DNA accumulates during life and has been postulated to lead to cancer and age-dependent diseases. Free radicals, especially OFR, are involved in multistage carcinogenesis, in both initiation and promotion. Antioxidant enzymes, the main scavengers of these radicals, are changed during carcinogenesis or after tumor formation. Low antioxidant enzymes will cause the accumulation of free radicals, which can result in damage to DNA, RNA, lipids, and proteins, and perhaps, finally lead to cancer (24).

Figure 1.4 shows that mechanism. Free radical species result from photochemical reactions and from oxidant stresses such as cigarette smoking and inflammation. Free radicals are produced by the cell such as macrophages or neutrophils. Neutrophils have the capability of damaging cells directly by two mechanisms, and indirectly by a third mechanism. One is the generation of oxygen free radicals, and the second is by the release of enzymes such as proteases. The prototype of the former mechanism is the “ischemia reperfusion injury”. Reperfusion delivers oxygen, allowing xanthine oxidase and catalase to produce superoxide anion and hydrogen peroxide. These reactive oxygen species can bind and damage essential cellular components such as membrane lipids and DNA. The indirect mechanisms is via
the formation of leukocyte aggregates in the microvasculature, thus exacerbating ischemic injury. Free radicals are capable of oxidizing DNA and RNA which may play a role of initiator in the carcinogenesis. Chronic infection generates free radical continuously. These free radicals may play an important role in the promotion of carcinogenesis. Free radicals initiate lipid peroxidation by reacting with polyunsaturated fatty acids and are capable of inactivating protein and enzymes by reactions with amino acids.

Figure 1.4: The role of free radicals in the pathogenesis of stomach cancer
Ohshima and Bartsch (25) developed a method that allows one to estimate the nitrosating potential in individuals by applying the \( N \)-nitrosopropoline test, in which the formation of \( N \)-nitrosopropoline is determined in the urine (26). \( N \)-nitrosopropoline is a harmless substance, however, its determination is thought to give an indication of the endogenous formation of \( N \)-nitroso-compounds in general. The test has been applied to urine from individuals in high- and low-risk areas of stomach cancer. Comparison within countries revealed a higher potential of forming \( N \)-nitrosopropoline in the high risk areas in Japan and Poland compared to low-risk areas (27). An interesting relationship of vitamin C metabolism to precancerous lesions for stomach cancer was found by Sobala (22), who showed that chronic gastritis diminishes the ability to secrete vitamin C into the interior of the stomach. This secretion is not connected with plasma vitamin C level or vitamin C intake. Currently it is not clear how this source of vitamin C affects, the vitamin C concentration in the stomach. An additional source of nitrosating agents was recently identified in bacteria and macrophages that produce nitric oxide, mostly from arginine (28). This source of nitrite formation may be important in local infection or inflammation (29). No convincing results have been presented to support the hypothesis that increased nitrate consumption in the general population results in increased nitrite formation, which then reacts with amines or amide substances to form carcinogens that induce stomach cancer. It seems that the endogenous formation of stomach cancer inducing or promoting substances is
complex and depends not only on the nitrite concentration but also on the availability of specific nitrosatable compounds. Exogenous nitrite/nitrate are found in the foods such as carrot, cauliflower, broccoli, cabbage, and radish. Amount of nitrites in foods varies, depending in part upon agricultural practice. Cured and smoked meats use nitrites as preservatives and additives. Endogenous nitrite is found in the saliva and in gastric juice, while N-nitrosocompounds are formed in the stomach by nitrosation from nitrite, nitrate, and urea. N-nitrosocompounds can cause DNA mutation.

1.4. Dietary factors influencing risk of gastric cancer

Diet appears to be involved in the early stages of transformation of normal cells into cancerous cells, with some factors acting as promoters and others as inhibitors. Three major etiologic factors are excessively salty foods and low intakes of ascorbic acid and carotene. Antioxidants such as ascorbic acid or β-carotene can inhibit nitrosamine formation. Once the cell is transformed into a cancerous cell, other nutritional factors, as yet unidentified, may either further promote or inhibit metastatic tumor growth. Preventing cancer initiation by inhibiting oxidation of RNA or DNA due to damage to guanine is another important cancer risk reduction strategy.

*Fresh vegetables and fruits*

A strong negative association between gastric cancer mortality and vegetable intake has been observed in several case-control studies. In the United States, per
capita vegetable intake has been increasing since at least 1930, paralleling the decline in gastric cancer mortality (30). The protective effect of high vegetable intake may be due either to a protective dietary pattern associated with high vegetable intake or to the presence of vegetable associated "anticarcinogens" such as vitamins A and C and the cruciferous associated antioxidants (31). Beta-carotene itself and its metabolites may play protective roles in preventing both the initiation and promotion of cancer. Beta-carotene neutralizes highly reactive free radicals that contain a nonpaired electron. Beta-carotene may also act to reduce cancer risk by stimulating cell-to-cell communication at gap junctions, and increasing control over the growth of precancerous cells. Ascorbic acid reacts with nitrite and converts it to nitrous oxide, while being oxidized itself to dehydroascorbic acid, thus preventing the formation of N-nitroso compounds by scavenging the nitrite precursor. Vitamin E has anticancer effects as a lipid antioxidant and free radical scavenger, blocking the formation of carcinogenic nitrosamines. The possible involvement of antioxidant nutrients in the pathogenesis of a stomach cancer is shown in Figure 1.5. Vitamin C, E and GSH (glutathione) can terminate free radical chain reactions or stabilize the free radicals by donating electrons or accepting electrons.

Vitamins

Consumption of fruits and vegetables is associated with an increased dietary intake of vitamin C, E, and A. Experimentally, all three vitamins have demonstrated the capacity to inhibit chemical processes associated with gastric carcinogenesis. In
animal and human studies, it has been shown that vitamin C inhibits intragastric formation of both nitrosamines from nitrite and amine precursors and nitrosomethylurea from sodium nitrite and methylurea. Vitamin E has been shown to be an important intracellular antioxidant and nitrite trapping agent and antitumorigenic properties. Vitamin A functions as an antioxidant and has demonstrated an ability to regulate cell differentiation, maintain host immunity, and prevent deterioration of the gastric mucus barrier.

![Diagram](attachment:image.png)

Figure 1.5: The role of antioxidants in the foods in the pathogenesis of stomach cancer
Glutathione (GSH)

Glutathione (γ-glutamylcysteinylglycine) is a tripeptide that exists either in a thiol (reduced, GSH) or a disulfide (oxidized, GSSG) form. Various mixed disulfides and thioethers account for a considerable share of the total glutathione pool (32). Glutathione is a ubiquitous and predominant nonprotein sulfhydryl compound in living organisms (33). It is a tripeptide, γ-glutamyl-L-cysteinyl-glycine, with an L-cysteinyl residue. Oxidation of glutathione may occur nonenzymatically or be associated with the activities of glutathione thiol transferase and of the glutathione peroxidases. The reaction catalyzed by glutathione disulfide reductase provides reducing power for the thiol transferases and peroxidases, and substrate for glutathione S-transferases. GSH appears to be synthesized in all mammalian cells and is normally maintained at millimolar concentrations (34). However, the synthetic capacity is insufficient to maintain GSH concentrations when tissues are exposed to certain drugs or their metabolites, redox cycling compounds, peroxides, X-rays, or ultraviolet radiation. Depletion of GSH impairs the ability of cells to protect against these agents and results in injury or death. Thus administration of supplemental GSH might allow the detoxification mechanisms to continue protecting cells from injury. Glutathione forms adduct with compounds of endogenous and exogenous origin: the glutamate residues of these S-conjugates are removed by the action of glutamyl transpeptidase. After cleavage of the corresponding dipeptide, acetylation converts the S-substituted compound to a mercapturate. The synthesis and degradation of glutathione follow the general pathway
of the γ-glutamyl cycle in which glutathione is synthesized intracellularly by the consecutive actions of γ-glutamylcysteine synthases and glutathione synthases; the process is feedback inhibited by glutathione. The breakdown of glutathione is initiated by the membrane-bound enzyme γ-glutamyl formation of γ-glutamyl-glutathione and hydrolysis of glutathione also occurs. The cysteinylglycine formed is split by membrane-bound dipeptidase, such activity is also found in cytosol (35). The level of glutathione differs markedly between different cell types and may also vary from one cell population or zone to another, as in liver, kidney and intestinal mucosa. Moreover, the cellular content of glutathione is also affected by a variety of other factors including age, nutritional status and exposure to certain xenobiotics. Glutathione is the most abundant low-molecular-weight thiol found in mammalian cells and functions in the metabolism of xenobiotics and carcinogens (36), in maintenance of the cellular thiol-to disulfide ratio (37), and in supply of a nontoxic reservoir of cysteine (38). In eukaryotic cells, most of the glutathione is present in thiol form in the cytosol, whereas the mitochondria contain a minor fraction of the cellular glutathione; this compartmentation may explain the observed existence of separate pools of glutathione with different turnover rates in liver.

Function of Glutathione

The function of cellular export of glutathione most certainly include the protection of the cell membrane against oxidative and other types of damage. Although
the levels of glutathione in blood plasma are normally very low. It is probable that the concentrations of glutathione in the interstitial fluid are greater than the peripheral plasma levels, thus reflecting the glutathione export process. It would be expected that the cell membrane would be protected by such exported extracellular glutathione. Export of glutathione may also provide a mechanism for the reduction of compounds that are in close proximity to the cell membrane, and might facilitate transport of certain compounds such as the components of some disulfides. Export of glutathione to membrane-bound transpeptidase leads to γ-glutamyl amino acid formation, part of a system for transport of amino acids including cysteine. Such transport from the liver, via plasma, to other tissues provides a mechanism for inter-organ distribution of cysteine moieties. The tripeptide glutathione (L-glutamyl-L-cysteinylglycine) participates in numerous cellular functions. Glutathione is involved, for example, in the synthesis of protein (39), regulation of enzyme activity (39), amino acid transport, and the catabolism of reactive oxygen species (40). Glutathione also serves as a coenzyme in the synthesis of endogenous compounds, thus, pathological causes by the action of chemical compounds are generally reflected by lowering of the concentration of the reduced form of glutathione, which can be accompanied by an increase in the levels of the oxidized form (GSSG). Therefore, monitoring of glutathione levels has been of growing importance in a wide variety of biological and medicinal fields (33).

Much evidence has accumulated pointing to the importance of GSH as a defense mechanism against damage induced by free radicals in many physiopathological
situations involving lipid peroxidation reactions. Reduced thiol agents, such as GSH, capable of interacting with free radicals to yield more stable elements and repair membrane lipid peroxides, may effectively be sufficient practical interment, particularly from the clinical point of view. Thus, the relationship between GSH and homeostatic balance of health has received considerable attention in recent years. Its implication in several severe diseases is under study by various investigators in the clinical and biochemical sciences (41-44).

Hepatic tissue contains a high level of glutathione, mainly in the reduced state. Its level decreases by about half during fasting, and increases again rapidly on refeeding (45). There are two pools of glutathione with turnover rates of about 2-h and 3-h (46). A large part of the hepatic glutathione is present in the cytosol and turns over very rapidly. Glutathione formation in the liver is closely related to nutritional conditions (47), especially the cysteine content of the diet (48), and from quantitative examinations of the relationship the cytosolic, content of the diet, and from quantitative examinations of this relationship the cytosolic, "labile" pool of glutathione was proposed to serve as a reservoir of cysteine in the liver (49).

Some epithelial cells have the capacity to take up exogenous GSH. Uptake by isolated cells of rat lung (type II) (50), renal proximal tubule (51), and small intestinal epithelium is Na⁺ dependent and inhibited by structure analogues of GSH. Detailed studies with vesicles from enterocytes and renal cortex showed that GSH transport occurred in the basolateral membranes and had characteristics of an electrogenic Na⁺
coupled symport system (52). Uptake of GSH by this system protected cells from injury by redox cycling agents or tert-butyl hydroperoxide (52). This indicates that methods to increase plasma GSH may be useful to prevent or treat certain types of toxicological and pathological processes.

Glutathione (L-γ-glutamyl-L-cystein + glycine) is synthesized within cells by the consecutive actions of γ-glutamylcysteine synthetase and glutathione synthetase:

\[
\text{L-glutamate + L-cysteine + ATP} \leftrightarrow \text{L-γ-glutamyl-L-cystein + ADP + Pi}
\]

\[
\text{L-γ-glutamyl-L-cystein + glycine + ATP} \leftrightarrow \text{glutathione + ADP + Pi}
\]

γ-glutamylcysteine synthetase is nonallosterically feedback-inhibited by glutathione. That is, glutathione normally regulates its own synthesis. Glutathione is present in most plant and animal tissues from which the human diet is derived. Much of this GSH is bioavailable because cells of the gastrointestinal tract have Na⁺-dependent and Na⁺-independent uptake mechanisms.

Glutathione has a number of cellular functions. It can function as an antioxidant in the ingesta, can maintain ascorbate in a reduced and functional form, can directly react with and inactivate toxic electrophiles in the diet, and can be broken down to yield cysteine. Studies have shown that dietary GSH enhances metabolic clearance of dietary peroxidized lipids and decreases their net absorption, and that consumption of foods high in GSH content is associated with about a 50% reduction in risk of oral and pharyngeal cancer (53). Of relevance to the subject matter of this volume, glutathione is an active participant in reactions that destroy hydrogen...
peroxide and organic peroxides, such as those catalyzed by glutathione peroxidase or certain glutathione S-transferases.

Glutathione peroxidase

Glutathione peroxidase (GPx) activity protects cell membranes from lipid peroxidation by the reduction of \( \text{H}_2\text{O}_2 \) and other hydroperoxides. Hydrogen peroxide is formed as a normal enzymatic product of numerous oxidase present in cell cytosol, microsome, peroxisomes and mitochondria. Hydrogen peroxide is formed by reduction of oxygen either directly in a two electron transfer reactions or via an initial one-electron step to \( \text{O}_2^- \) (superoxide) followed by dismutase to \( \text{H}_2\text{O}_2 \). Hydrogen peroxide has useful functions such as in phagocytosis, ethanol and methanol oxidation, and thyroid hormone production(54). The toxic action of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) is due to their capacity to generate other reactive oxygen species such as the hydroxyl radical and singlet oxygen, which can then initiate a radical chain reaction, leading to extensive lipid and organic peroxide formation. The initial steps in lipoperoxide formation are hydrogen abstraction from a polyunsaturated fatty acid to form a free radical, subsequent diene conjugation, and formation of a peroxide radical by incorporation of molecular oxygen. Lipid peroxidation can affect membrane bound enzyme systems and membrane permeability(55).
Glutathione Reductase

Glutathione reductase catalyzes the reduction of glutathione disulfide (GSSG) to GSH; a reaction which is of fundamental importance in maintaining the concentration of intracellular GSH for other reactions. GSSG is produced in the GSH peroxidase reaction and free radical reducing reactions, where GSH is serving a major function as a reductant in oxidation-reduction processes.

The oxidized form of glutathione may be converted back to the reduced form by the activity of the enzyme glutathione reductase, which catalyses the reaction given in the following equation.

\[ \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+ \]

The ubiquitous tripeptide glutathione (GSH), which is the most abundant low molecular weight thiol in almost all cells, is involved in a wide range of enzymatic reactions. A major function of GSH is to serve as a reductant in oxidation-reduction processes; a function resulting in the formation of glutathione disulfide (GSSG). It is well established that numerous additional reactions utilize glutathione in the reduced form, and the reduction of GSSG is consequently of fundamental importance for the metabolic function of glutathione. The mammalian liver is among the most GSH-dependent biochemical reaction sites. Therefore, it is of special significance to investigate the enzymatic reduction of GSSG in the liver (56).
Glutathione S-Transferase

GSH S-transferases (RX: glutathione S-transferase-GST EC 2.5.1.18) are enzymes catalyzing the conjugation of GSH with electrophilic and potential alkylating agents, which is the first step in mercapturic acid formation (57). Hence these enzymes play an important role in the detoxification of xenobiotics and their reactive metabolites, which can be generated by metabolic activation under influence of the microsomal cytochrome P450 containing mixed function oxidase system. The general reaction catalyzed by the GSTs, the conjugation of xenobiotics with reduced glutathione, is important in the handling of electrophilic carcinogens because it may prevent their binding to DNA. While the isoenzymes encoded by the different GST genes have common substrates, the conjugation of some electrophiles is preferentially catalyzed by the isoforms of only one class. For example, the conjugation of potentially cyto- and genotoxic epoxides such as styrene 7,8-oxide is more effectively catalyzed by μ (mu) class enzymes than by those of the α (alpha) or π (pi) classes (58); the isozymes in human tissues can be usefully classified into the α, μ and π classes. For example, the conjugation of potentially cyto- and genotoxic epoxides such as styrene 7,8-oxide σ (si) more effectively catalyzed by μ class enzymes than by those of the alpha or π classes (59). These enzymes catalyze the reaction of such compounds with the -SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. Glutathione conjugates are thought to be metabolized further by cleavage of the glutamate and glycine residues,
followed by acetylation of the resultant free amino group of the cysteine residue, to produce the final product, a mercapturic acid. The mercapturic acids, i.e. S-alkylated derivatives of N-acetylcysteine, are excreted. These enzymes play an important role in the detoxification of xenobiotics and their reactive metabolites, which can be generated by metabolic activation under influence of the microsomal cytochrome P450.

Figure 1.6 shows the mechanism to prevent stomach cancer from free radical attack. High intake of vitamin C, E, β-carotene, and glutathione can prevent the pathogenesis of the stomach cancer and boost first line defense system against free radical attack. Superoxide dismutase, catalase, and glutathione peroxidase remove superoxide anions and hydrogen peroxides.
1.5. Kimchi

Kimchi (pickled vegetable) has been consumed since the 3rd or 4th century A.D. in Korea (60). Kimchi is a traditional, fermented Korean food that is prepared through a series of processes, including pretreatment of oriental cabbage, blending with various spices and other ingredients, such as green onion, garlic, red pepper powder, shrimp sauce, and ginger, and fermentation over time. Fermented vegetables are consumed worldwide such as sauerkraut, Chinese *humchoy*, Japanese *zukemono*, in addition to Kimchi (61). It should be noted that even though Kimchi is prepared by a fermentation process similar to that of sauerkraut or fermented vegetables of China, Kimchi has some distinctive characteristics; there are more than 150 different kinds of Kimchi, depending on the raw materials and preparation methods. Kimchi liquid is
also consumed with whole Kimchi. Although properly fermented (ripened) Kimchi is
the type mainly consumed, relatively fresh Kimchi (unripened) and sour Kimchi
(overripened) are also consumed. The characteristics of Kimchi differ depending on
the Kimchi varieties, raw materials used, process, fermentation, and preservation
methods. Kimchi fermentation is initiated by various microorganisms that are
originally present in the raw materials (62), but the fermentation process is generally
dominated by lactic acid bacteria. Numerous physiochemical and biological factors
influence the fermentation, growth, and sequential appearance of principal
microorganisms involved in the fermentation. Complex biochemical changes occur
depending on the environmental conditions before, during and after fermentation. The
most important characteristics are the compositional changes of sugars and vitamins,
formation and accumulation of organic acids, and texture degradation and softening.
Kimchi is an important source of vitamins, minerals, dietary fiber, and other nutrients
to Koreans during the winter. Nutritional compositions of Kimchi vary depending on
the varieties of Kimchi. But the nutritional composition of a representative Kimchi is
that: moisture - 88.4 g/100g, crude protein - 2 g/100g, crude lipid - 0.6 g/100g, total
sugar - 1.3 g/100g, crude fiber - 7.2 g/100g, crude ash - 0.5 g/100g, calcium - 28
mg/100 g, iron-trace, Vitamin A (IU) - 492/100g, Vitamin B₁ (mg) - 0.03/100 g,
vitamin B₂ (mg) - 0.06/100g, niacin -2.1 mg/100g, and vitamin C (mg) - 12/100 g
(Table 1) (63). According to a national nutrition survey in Koreas, a Korean adult
consumes 50-100g of Kimchi/day in summer and 150-200 g/day in winter, which
constitutes 12.5% of total daily food intake (64). Due to high spice, salts and other ingredients, it is possible that Kimchi inhibits the free radical defense enzymes in the stomach. Figure 1.7 shows the role of Kimchi in the function of antioxidant enzymes in the stomach mucosa.
<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Ingredient Percentage</th>
<th>Raw materials</th>
<th>Ingredient Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese cabbage</td>
<td>80.0</td>
<td>Mustard leaves</td>
<td>1.0</td>
</tr>
<tr>
<td>Radish</td>
<td>12.0</td>
<td>Parsley</td>
<td>1.0</td>
</tr>
<tr>
<td>Red pepper</td>
<td>1.0</td>
<td>Pickled shrimp</td>
<td>0.8</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.5</td>
<td>Fermented fish</td>
<td>1.1</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.4</td>
<td>Salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Green onion</td>
<td>1.0</td>
<td>Others</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 1.1: Typical ingredients of Kimchi
Figure 1.7: The role of Kimchi in the function of antioxidant enzymes in the mucous
Spicy food

Red pepper, *Capsicum longum*, commonly called “chili”, is a member of the Solanaceae Family. Since its introduction to Korea in the beginning of the seventeenth century, it has been used in almost all kinds of Korean dishes. Red pepper has twice as much vitamin C as orange does, therefore red pepper is an important source of vitamins during wintertime (64). Chili helps the lactic fermentation process that leads to tasty Kimchi, and also prevents the fish juice from turning sour.

Capsaicin, a main component of red pepper, facilitates the secretion of the pseudo-hormonic substances like endorphin, which helps remove nicotine from the surface of lung. However, capsaicin, the pungent of hot red peppers (Capsicum), may be carcinogenic. In mice, capsaicin has been shown to induce duodenal adenocarcinomas to act as a promoter of stomach and liver tumors, and to induce micronuclei formation. *In vitro*, capsaicin was mutagenic to Chinese hamster lung cells and exhibited mutagenic activity in the Ames test. High mortality of gastric cancer in Mexico, which was 10% of cancer death in 1990 may be attributable to high consumption of chili, 20 g/d/capita (one chili pepper/d/capita) (65).

Salt

High salt concentration may enhance gastric carcinogenesis. Mice fed a diet of dried cod containing 7% sodium chloride developed both acute and chronic gastritis. In humans, consumption of pickled in high-salt soysauce led to abnormal changes in
gastric mucosa (66). Rats fed salty rice demonstrated hyperplasia of the forestomach and atrophy of the glandular stomach (67). Chronic atrophic gastritis and intestinal metaplasia each represent precursors of human gastric cancer, and evidence indicates that the incidence of those gastric changes increases in parallel with an increase of gastric cancer risk (68). The establishment of gastric atrophy, with a resultant reduction of parietal cells as well as of mucous cells, provides a favorable environment not only for the formation of carcinogenic nitroso compounds in the stomach, but also for the access of the formed carcinogen to the target cells.

Carbohydrate

The inverse association between gastric cancer risk and socioeconomic status led to the hypothesis that high carbohydrate intake at an early age predisposes to increased gastric cancer risk. A high starch diet, comprised predominantly of whole grain cereals, may mechanically abrade the stomach mucosa, thus promoting absorption of a carcinogen (69). In addition, a high carbohydrate intake has been shown to be inversely associated with protein and fat intake (70). Low-protein diets have been shown to inhibit repair of gastric lesions by reducing gastric mucus production, facilitating absorption of a potential gastric carcinogen (71). Also high-starch/low-protein diets may reduce the concentration of agents in gastric juice that actively break down nitrites.
CHAPTER 2

OBJECTIVES AND RATIONALE

Stomach cancer was the most common cancer around the world in the first half of this century, and is now characterized by a rapid decrease in incidence in North America and Western Europe. However, in other regions such as Asia, Latin America and Eastern Europe, incidence of stomach cancer remains high (72). Korea is one of the countries with the highest incidence of stomach cancer in the world, e.g. the age-standardized annual incidence of stomach cancer is 57.9 % per 100,000 for males and 26.1 % per 100,000 for females (12). In contrast, stomach cancer mortality is rare in Korean-Americans resident in Ohio (73). The number of Korean-Americans residents in Ohio is 10,830. Only 12 out of 820 reported stomach cancer cases in Ohio are Asian, and the nationality of these Asians is unknown.

The specific cause for the high incidence of stomach cancer in Korea is still unknown. However, epidemiological studies demonstrated a significant difference in cancer incidence within a country and among countries as well as a difference in mortality rates between first and second generation migrants. From these findings, it
is reasonable to assume that environmental factors, particularly dietary factors, have the most important etiologic implications for stomach cancer (8). Thus, some unique Korean food frequently and consistently consumed in Korea may be responsible for the high incidence of stomach cancer. In search of the dietary factor(s), it is reported by 1990 Korean National Nutrition Survey data (74) that the most popular and frequent food of Koreans is Kimchi which is often consumed with rice and soup or with noodles. Kimchi, a fermented spicy and salty vegetable, is a traditional Korean food. Oriental cabbage or radish is the most popular major raw material for Kimchi, the cabbage or radish is salted after prebrining, blended with various hot spices and other minor ingredients, and then fermented (63).

Due to the fermentation process, the high spicy content and the high salt content, Kimchi may be a contributing factor for promoting the high incidence of stomach cancer. One of the mechanisms by which Kimchi may promote carcinogenesis is to weaken the oxygen free radical defense enzymes in the mucosal lining cells in the stomach. Oxygen free radicals are undesirable but inevitable byproducts of aerobic metabolism (75). They are formed continuously in vivo by oxidative cellular metabolism, by phagocytes in response to microbial infection and inflammation, as well as by metabolic activation of xenobiotics. For example, Helicobacter pylori (76), a gram-negative, microaerophilic, spiral bacteria commonly present in the stomach of patients with chronic gastritis, is being actively explored as a risk factor for gastric carcinoma due to its ability to cause excess oxygen radicals. In
addition, the stomach is the organ of the first prolonged contact and exposure to ingested food containing foreign compounds such as carcinogens. The detoxification enzyme system of the stomach is also critically important to detoxify carcinogens.

The hypothesis of the research project is that Kimchi is a promoter of stomach cancer by decreasing the oxygen free radical enzymatic defense system and detoxification enzymes in the stomach. Thus, the objective of the experiment was to determine the effect of dietary Kimchi on the activities of oxygen free radical defense enzymes; glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R), and the detoxification enzyme; glutathione S-transferase (GST), and total glutathione content in the stomach of the Wistar rats.
CHAPTER 3

COMPARISON OF DIETARY PATTERNS OF KOREAN-AMERICANS AND KOREANS AND THEIR POSSIBLE CAUSATIVE RELATIONSHIPS WITH STOMACH CANCER
INTRODUCTION

Gastric cancer kills over a half-million people in the world every year. Although gastric cancer is considered a multifactorial disease, most epidemiology studies have focused on diet as the overriding etiologic factor (72).

Stomach cancer, which can be benign or malignant, may arise from the epithelium and, less commonly, from the muscle, nerves, and lymphoid tissue of the stomach wall. Gastric cancer is not histologically homogeneous. The histologic structure of gastric carcinoma often displays the characteristics of intestinal mucosa. The two major histologic types of gastric cancer are intestinal and diffuse. Intestinal type gastric cancer is more prevalent in males and in older age groups. The diffuse type, on the other hand, occurs at a similar ratio and occurs more frequently in younger age groups. It appears that diffuse type gastric cancer, with its preference for younger individuals and its association with blood group A, is more closely governed by genetic factors than by environmental factors. Contrarily, the intestinal type seems to be more closely associated with environmental factors. Chronic atrophic, gastritis and its associated lesion, and intestinal metaplasia, are the precursors of intestinal type gastric cancer. The rapid decline in stomach cancer mortality rates in the United States, thus, suggest that the major etiologic factors in gastric cancer are environmental rather than genetic (77).

Stomach cancer is the most common malignant neoplasm among Koreans, and the age-adjusted annual incidence of stomach cancer is the highest in the world: 57.9
per 100,000 per males and 25.1 per 100,000 for females over the period 1986-1987 (12). It was reported the point prevalence rate to be 74 per 100,000 in males and 45 per 100,000 in females (78). It accounts for 30 percent of male cancer and 17 percent of female cancer according to the report of the Korean Ministry of Health and Social Affairs in 1989 (79). Although the incidence and mortality rate of stomach cancer have been decreasing in the United States over the last 50 years, it remains as the most commonly occurring cancer and the number one cause of cancer death in Korea. In Los Angeles County, gastric cancer incidence is greater by factor of 5 in Korean males (44.8 cases per 100,000) than in white males (8.6 cases per 100,000) (80). Korean-Americans in Illinois showed a higher rate of stomach cancer than African-Americans and Caucasian-Americans, indicating that profound interactions existed between genetic and environmental factors (81).

In Ohio, first-generation immigrants to areas with lower gastric cancer rates show some diminution of risk. This suggests that immigrants are either selectively less prone to developing gastric cancer, or that gastric cancer risk may be modified to some extent in later life stage. If the latter is true, identification of the modifying mechanism(s) may provide another possibility for prevention in high-risk individuals by removal of a potentiating factors or introduction of a protective factors.

Therefore, this Korean and Korean-American dietary comparison was required to study prevention in high-risk individuals.
MATERIALS AND METHODS

Research Design

This study, using the descriptive survey research approach, was designed to describe differences in dietary practices between Korean immigrants in central Ohio and Koreans in Korea, and to compare the incidence of stomach cancer in each group. Data was gathered on a 2-day dietary record. The time period of April 1996 to December 1996 was utilized for planning, sampling, development of the instrument, questionnaire distribution, data collection, data analysis, and interpretation.

Population Sample

Subjects from the United States were randomly selected from Korean church members in Columbus, Ohio and the Columbus Korean-Americans Association directory. In Korea, subjects were randomly selected from the phone directory in Seoul, Korea. Three hundred survey forms were sent out both in the United States and in Korea. Subjects were males and females, 35 years of age and older. Two-day dietary records with a brief health history were required from all subjects.

Instrument

A structured, self-administered questionnaire was developed by the researcher and approved by an expert panel, consisting of faculty members from the Human Nutrition and Food Management Department at The Ohio State University. The questionnaire was piloted, using 20 Korean-American immigrants. The questionnaire
was further revised to maximize validity and clarity. The content of the questionnaires was very similar in Columbus and Seoul. Korean language was used in the questionnaire.

The questionnaire consisted of two parts. Part I assessed demographics, dietary practices, and health conditions related to stomach disease and stomach cancer. Subjects were asked their age, gender, area of residence, occupation, and highest level of education. Part II was a 2-day weighted dietary record during weekday. In the pilot study, respondents indicated that the two consecutive day dietary record was enough to see the dietary difference, because Korean meals usually consist of rice, soup or stew, Kimchi and other side dishes. Side dishes changed every other day and the third day left overs were eaten.

Data Collection

A cover letter stating the study's objectives and goals was mailed out to each Korean-American resident in Columbus and its vicinity and to Koreans in Seoul and its vicinity. Self-addressed and stamped envelopes were enclosed for responses.

After six weeks, postcard reminders were mailed out to non-respondents and/or phone call reminders were made to local non-respondents. Extra copies of the questionnaires and return envelopes were sent out as needed.
Data Analyses

All returned complete questionnaires were used as sources of data. Data from Part I of the questionnaires were numerically coded. The data were then analyzed through the SAS® (release 6.07, 1989, SAS Institute, Cary, NC), utilizing the facilities and consultation in Academic Computing Service at The Ohio State University. Data from part II of the questionnaires were analyzed using Food Processor Nutrition Analysis Software® for nutrients.

RESULTS

Response Rate

Of 300 questionnaires mailed out each in Columbus and in Seoul, 244 questionnaires were returned from Columbus, and 213 questionnaires from Seoul. Response rates were 81 and 71 %, respectively.
<table>
<thead>
<tr>
<th></th>
<th>Korean-American</th>
<th>Korean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>89 (36.5%)</td>
<td>114 (53.5%)</td>
</tr>
<tr>
<td>female</td>
<td>155 (63.5%)</td>
<td>99 (46.5%)</td>
</tr>
<tr>
<td><strong>Age (year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>28 (11.5%)</td>
<td>65 (30.5%)</td>
</tr>
<tr>
<td>41-50</td>
<td>80 (32.8%)</td>
<td>48 (22.5%)</td>
</tr>
<tr>
<td>51-60</td>
<td>84 (34.4%)</td>
<td>24 (11.3%)</td>
</tr>
<tr>
<td>61-70</td>
<td>31 (12.7%)</td>
<td>20 (9.4%)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>21 (8.6%)</td>
<td>56 (26.2%)</td>
</tr>
<tr>
<td><strong>Place</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>big city</td>
<td>172 (70.49%)</td>
<td>87 (40.85%)</td>
</tr>
<tr>
<td>medium city</td>
<td>58 (23.77%)</td>
<td>49 (23.0%)</td>
</tr>
<tr>
<td>small city</td>
<td>12 (4.92%)</td>
<td>73 (34.27%)</td>
</tr>
<tr>
<td>town</td>
<td>2 (0.82%)</td>
<td>4 (1.88%)</td>
</tr>
<tr>
<td><strong>Highest Level of Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6 grade</td>
<td>20 (8.2%)</td>
<td>23 (10.8%)</td>
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<tr>
<td>7-9 grade</td>
<td>19 (7.79%)</td>
<td>40 (18.78%)</td>
</tr>
<tr>
<td>10-12 grade</td>
<td>54 (22.13%)</td>
<td>71 (33.33%)</td>
</tr>
<tr>
<td>13-16 grade</td>
<td>103 (42.21%)</td>
<td>68 (31.92%)</td>
</tr>
<tr>
<td>&gt;16 grade</td>
<td>48 (19.67%)</td>
<td>11 (5.16%)</td>
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<td><strong>Job</strong></td>
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</tr>
<tr>
<td>professional</td>
<td>37 (15.1%)</td>
<td>3 (0.14%)</td>
</tr>
<tr>
<td>officer</td>
<td>46 (18.9%)</td>
<td>54 (25.3%)</td>
</tr>
<tr>
<td>self-employ</td>
<td>53 (21.7%)</td>
<td>79 (37.1%)</td>
</tr>
<tr>
<td>housewife</td>
<td>62 (25.4%)</td>
<td>55 (25.8%)</td>
</tr>
<tr>
<td>others</td>
<td>46 (18.9%)</td>
<td>22 (10.3%)</td>
</tr>
</tbody>
</table>

Table 3.1: Demographic characteristics of study subjects
Table 3.1 explained the demographic characteristics of the two groups according to gender, age, place of residence, the highest level of education and job. Among Korean-Americans, more females responded than males did (63.5 % vs. 36.5 %), while among Koreans, more males responded than females did (53.5 % vs. 46.5 %), even though there was no statistical significance in gender of respondents.

And, mean age of the two groups was 50.4 years old, but the age distribution between two groups was very different. That is, majority of Korean-Americans respondents were between 41 and 60 years old, while majority of Koreans respondents were at their 30's and > 70 years old.

A majority of the Korean-Americans respondents (70.5 %) lived in a big city, whereas Koreans respondents were evenly distributed in big, medium, and small cities (40.9, 23.0 and 34.3 % respectively). However, it seems Koreans respondents misinterpreted the size of their places because the questionnaires were distributed mainly in Seoul whose population is almost 10 times that of Columbus.

There were significant differences in education level and jobs between Koreans and Korean-Americans respondents. Majority of Koreans respondents had 12 years of education or less (62.9 %), while 61.8 % of Korean-American respondents had more than 13 years of education. The tendency of education level was reflected in their jobs. More Korean-American respondents were in professional jobs than Koreans respondents (15.1 % vs. 0.14 %).
<table>
<thead>
<tr>
<th></th>
<th>Korean-American</th>
<th>Korean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>23.77 (±2.89)</td>
<td>22.96 (±2.32)</td>
</tr>
<tr>
<td>female</td>
<td>22.53 (±3.34)</td>
<td>21.5 (±2.15)</td>
</tr>
<tr>
<td><strong>Cigarette use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male+female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>current</td>
<td>22 (24.7%) + 8 (5.1%)</td>
<td>70 (61.4%) + 12 (12.1%)</td>
</tr>
<tr>
<td>never</td>
<td>30 (33.7%) + 140 (90.3%)</td>
<td>24 (21.1%) + 84 (84.9%)</td>
</tr>
<tr>
<td>quit</td>
<td>37 (41.6%) + 7 (4.5%)</td>
<td>20 (17.5%) + 3 (3.0%)</td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male+female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>current</td>
<td>42 (47.2%) + 20 (12.9%)</td>
<td>57 (50%) + 43 (43.4%)</td>
</tr>
<tr>
<td>never</td>
<td>30 (33.7%) + 131 (84.5%)</td>
<td>26 (22.8%) + 52 (52.5%)</td>
</tr>
<tr>
<td>quit</td>
<td>17 (19.1%) + 4 (2.6%)</td>
<td>31 (27.2%) + 4 (4.1%)</td>
</tr>
<tr>
<td><strong>supplementation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vitamin</td>
<td>102 (41.80%)</td>
<td>25 (11.74%)</td>
</tr>
<tr>
<td>mineral</td>
<td>4 (1.64%)</td>
<td>2 (0.94%)</td>
</tr>
<tr>
<td>aloe vera</td>
<td>0 (0%)</td>
<td>5 (2.35%)</td>
</tr>
<tr>
<td>royal jelly</td>
<td>13 (5.33%)</td>
<td>5 (2.35%)</td>
</tr>
<tr>
<td>ginseng</td>
<td>10 (4.10%)</td>
<td>5 (2.35%)</td>
</tr>
<tr>
<td>other</td>
<td>5 (2.05%)</td>
<td>5 (2.35%)</td>
</tr>
<tr>
<td>none</td>
<td>110 (45.08%)</td>
<td>166 (77.93%)</td>
</tr>
<tr>
<td><strong>disease type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>188 (77.0 %)</td>
<td>169 (79.3%)</td>
</tr>
<tr>
<td>heart condition</td>
<td>5 (2.0 %)</td>
<td>3 (1.4%)</td>
</tr>
<tr>
<td>obesity</td>
<td>2 (0.8 %)</td>
<td>8 (3.8%)</td>
</tr>
<tr>
<td>high blood pressure</td>
<td>17 (7.0 %)</td>
<td>12 (5.6%)</td>
</tr>
<tr>
<td>diabetes</td>
<td>14 (5.73 %)</td>
<td>6 (2.8%)</td>
</tr>
<tr>
<td>cancer</td>
<td>1 (0.4%)</td>
<td>6 (2.8%)</td>
</tr>
<tr>
<td>others</td>
<td>17 (6.97%)</td>
<td>9 (4.2%)</td>
</tr>
<tr>
<td><strong>stomach disease experience</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stomach ulcer</td>
<td>24 (9.84 %)</td>
<td>26 (12.21 %)</td>
</tr>
<tr>
<td>heart burn</td>
<td>38 (15.57 %)</td>
<td>40 (18.78 %)</td>
</tr>
<tr>
<td>gastroenteritis</td>
<td>20 (8.17%)</td>
<td>24 (11.27 %)</td>
</tr>
<tr>
<td>none</td>
<td>162 (66.39 %)</td>
<td>123 (57.75 %)</td>
</tr>
<tr>
<td><strong>Family history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>35 (14.34 %)</td>
<td>41 (19.25 %)</td>
</tr>
<tr>
<td>no</td>
<td>209 (85.66 %)</td>
<td>172 (80.75 %)</td>
</tr>
<tr>
<td><strong>Are you on a diet?</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>59 (24.18%)</td>
<td>27 (12.68 %)</td>
</tr>
<tr>
<td>no</td>
<td>185 (75.28 %)</td>
<td>186 (87.32 %)</td>
</tr>
</tbody>
</table>

Table 3.2: Characteristics of study subjects in Columbus, OH and Seoul, Korea
Table 3.2. There were no statistical differences in BMI between the two groups, which ranged from 21.5 to 23.8. There were significant differences in cigarette smoking and alcohol consumption. Both in cigarette smoking and alcohol consumption, Koreans showed higher levels of consumption than Korean-Americans did. However, the gender difference in alcohol and cigarette consumption within a group showed the same trend. Korean-Americans intake significant higher percentages of vitamin, mineral, royal jelly and ginseng supplements than did Koreans. However, Koreans more consumed aloe vera than did Korean-Americans. Most conspicuous difference was vitamin intake, in which 41.8 % of the Korean-Americans took vitamins while only 11.7 % of the Koreans took vitamins. There were no differences in disease type, medication related to stomach disease, and family history between Korean-Americans and Koreans.
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>K-A Male</th>
<th>K Male</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy, kcal</td>
<td>2304.1±242.3</td>
<td>2097.8±209.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein, g</td>
<td>109.1±29.8</td>
<td>63.1±17.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animal protein, g</td>
<td>48.7±6.7</td>
<td>30.8±4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>259.3±40.2</td>
<td>309.1±48.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid, g</td>
<td>97.9±21.7</td>
<td>62.8±20.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monounsaturated, %</td>
<td>11.9±2.0</td>
<td>8.9±2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polyunsaturated, %</td>
<td>12.8±3.8</td>
<td>10.9±4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saturated, %</td>
<td>8.3±1.9</td>
<td>5.8±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>144.4±81.3</td>
<td>97.6±58.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>30.3±11.7</td>
<td>23.6±12.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Glutathione, mg</td>
<td>35.4±1.7</td>
<td>28.7±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zn, mg</td>
<td>13.6±4.4</td>
<td>8.4±2.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cu, mg</td>
<td>2.2±1.0</td>
<td>1.2±0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fe, mg</td>
<td>28.8±9.8</td>
<td>21.1±3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NaCl, g</td>
<td>2.87±1.4</td>
<td>3.37±0.9</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Table 3.3: Comparison of daily nutrients ingested by Korean-American male and Korean male subjects
The daily nutrients consumed by Korean-American males and Korean males were compared as shown in Table 3. Korean-American males consumed more nutrients than Korean males did except for carbohydrates and salts. Korean-American males consumed significantly higher antioxidants including vitamin C, vitamin E, and glutathione than did Korean males, Korean-American males consumed a total of 2304 kcal of energy per day, which was evenly distributed from protein, carbohydrate and fat, while Korean males consumed total 2097 kcal of energy per day, which was mainly from carbohydrates. Korean-American males consumed protein from animal sources (Figure 3.5), while Korean males' protein sources were vegetables and soybeans. Even though the total intake of lipid (Figure 3.6, 3.7, and 3.8) was much higher in Korean-American males, the ratio of lipid composition, as monounsaturated, polyunsaturated and saturated fats, was similar between the two groups. In addition, Korean-American males consumed more glutathione (Figure 3.1), zinc (Figure 3.2), iron (Figure 3.3), and copper (Figure 3.4) than did Korean male.
Figure 3.1: The comparison of glutathione intake between Korean-American male and Korean male subjects
Figure 3.2: The comparison of zinc intake between Korean-American male and Korean male subjects
Figure 3.3: The comparison of iron intake between Korean-American male and Korean male subjects
Figure 3.4: The comparison of copper intake between Korean-American male and Korean male subjects
Figure 3.5: The comparison of Animal protein intake between Korean-American male and Korean male subjects
Figure 3.6: The comparison of saturated fatty acid intake between Korean-American male and Korean male subjects
Figure 3.7: The comparison of monounsaturated fatty acid intake between Korean-American and Korean male subjects.
Figure 3.8: The comparison of polyunsaturated fatty acid intake between Korean-American and Korean male subjects
<table>
<thead>
<tr>
<th>nutrient</th>
<th>K-A Female</th>
<th>K Female</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy, kcal</td>
<td>1776.5±213.7</td>
<td>1893.2±148.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein, g</td>
<td>94.5±27.7</td>
<td>65.6±16.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animal protein, g</td>
<td>50.2±5.2</td>
<td>22.7±3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>211.9±44.5</td>
<td>296.1±35.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid, g</td>
<td>66.9±16.8</td>
<td>49.9±13.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monounsaturated, %</td>
<td>9.6±2.4</td>
<td>7.5±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polyunsaturated, %</td>
<td>9.6±2.1</td>
<td>7.7±3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saturated, %</td>
<td>7.4±2.4</td>
<td>5.7±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>159.5±15.4</td>
<td>205.5±10.0</td>
<td>0.724</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>19.4±9.9</td>
<td>14.7±7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glutathione, mg</td>
<td>33.3±1.10</td>
<td>23.1±0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zn, mg</td>
<td>12.2±4.32</td>
<td>8.6±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cu, mg</td>
<td>2.1±1.1</td>
<td>1.2±0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fe, mg</td>
<td>23.9±10.5</td>
<td>21.3±3.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NaCl, g</td>
<td>2.29±1.2</td>
<td>3.57±1.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3.4: Comparison of daily nutrients ingested by Korean-American female and Korean female subjects
Table 3.4 shows the difference in intake of macronutrients and micronutrients between Korean-American females and Korean females. Female groups had almost the same trends as the male groups did except for the total energy consumption. Korean females consumed higher total energy than did Korean-American females (1776.5 kcal vs. 1893.2 kcal). There were differences in energy sources between Korean-American females and Korean females.

The main energy sources of Korean-American females were protein (21%), carbohydrate (48%), and lipid (31%), while Korean females energy sources were protein (13%), carbohydrate (63%), and lipid (24%). Also, there were significant differences in protein sources. That is, Korean-American females protein sources were mainly animal protein, while protein sources of Korean females were vegetables such as beans. In composition of lipid intake, the two groups showed the same trend: monounsaturated, polyunsaturated, and saturated (10:10:10). Korean females consumed more Vitamin C and salt than did Korean-American females. Other nutrients such as vitamin E, glutathione, Zn, Cu, and iron were consumed by Korean-American females more than by Korean females.
Figure 3.9: Intake of major dietary factors between Koreans and Korean-Americans
Figure 3.9 shows the dietary pattern differences between Koreans and Korean-Americans. Two groups showed significant differences in Kimchi intake. Koreans ate more than 60 g of Kimchi per day, whereas Korean-Americans consumed 20 g of Kimchi per day. Significant differences were also found in bean paste and pickled fish intakes; Koreans ate more pickled fish and bean paste stew or soup. Conversely, Korean-Americans consumed more seaweed than Koreans did. Half of Korean-Americans respondents consumed 1 cup of brown rice or 200 g of medium grain no-enriched rice per day per person, while most of Korean respondents consumed 2 cups of medium grain regular rice a day. Only a few Koreans consumed brown rice as a healthy food.
<table>
<thead>
<tr>
<th>Duration of residence in the United States (years)</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 10</td>
<td>56</td>
<td>23.0 %</td>
</tr>
<tr>
<td>11 - 20</td>
<td>102</td>
<td>41.8 %</td>
</tr>
<tr>
<td>21 - 30</td>
<td>70</td>
<td>28.7 %</td>
</tr>
<tr>
<td>31 - 40</td>
<td>14</td>
<td>5.7 %</td>
</tr>
<tr>
<td>&gt; 41</td>
<td>2</td>
<td>0.8 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preference of food</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korean food</td>
<td>158</td>
<td>65.6 %</td>
</tr>
<tr>
<td>American food</td>
<td>43</td>
<td>16.8 %</td>
</tr>
<tr>
<td>Same</td>
<td>43</td>
<td>17.6 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency of non-Korean ingredient used</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>never</td>
<td>46</td>
<td>18.9 %</td>
</tr>
<tr>
<td>some of time</td>
<td>139</td>
<td>57.0 %</td>
</tr>
<tr>
<td>most of time</td>
<td>32</td>
<td>13.1 %</td>
</tr>
<tr>
<td>all the time</td>
<td>27</td>
<td>11.1 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency of traditional cooking method</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>all the time</td>
<td>53</td>
<td>21.8 %</td>
</tr>
<tr>
<td>never</td>
<td>84</td>
<td>34.6 %</td>
</tr>
<tr>
<td>most of time</td>
<td>87</td>
<td>35.8 %</td>
</tr>
<tr>
<td>some time</td>
<td>19</td>
<td>7.8 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modification of preparing and cooking</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>spice</td>
<td>135</td>
<td>67.7 %</td>
</tr>
<tr>
<td>main ingredient</td>
<td>49</td>
<td>11.5 %</td>
</tr>
<tr>
<td>reduce fat</td>
<td>80</td>
<td>19.3 %</td>
</tr>
<tr>
<td>different type stew</td>
<td>8</td>
<td>1.6 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eating habit change</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>197</td>
<td>80.7 %</td>
</tr>
<tr>
<td>no</td>
<td>47</td>
<td>19.3 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food habit change (based on previous question)</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>salty food avoid</td>
<td>154</td>
<td>46.4 %</td>
</tr>
<tr>
<td>spicy food avoid</td>
<td>149</td>
<td>44.9 %</td>
</tr>
<tr>
<td>hot food avoid</td>
<td>7</td>
<td>2.1 %</td>
</tr>
<tr>
<td>sweet food favorite</td>
<td>22</td>
<td>6.6 %</td>
</tr>
</tbody>
</table>

Table 3.5: Characteristics of Korean-Americans (to be continued)
Table 3.5: Characteristics of Korean-Americans

<table>
<thead>
<tr>
<th>Duration of residence and preference of food</th>
<th>Duration (year)</th>
<th>Korean Food</th>
<th>American Food</th>
<th>Same</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>41</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>73</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>41</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>31-40</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;41</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of residence and frequencies of using non-Korean ingredients</th>
<th>Duration (years)</th>
<th>never</th>
<th>some</th>
<th>most</th>
<th>all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>13</td>
<td>33</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>20</td>
<td>66</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>12</td>
<td>34</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>31-40</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>41-50</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3.5 indicates Korean-Americans have changed dietary habits since they moved to America. Seventy percent of Korean-Americans respondents have been living in America more than 10 years to 30 years. Sixty five percent of Korean-Americans respondents showed that they preferred Korean food. However, it was found that cooking methods or ingredients used were modified, and only 20 % of respondents kept traditional cooking methods using Korean ingredients. Korean-Americans were also found to have changed their food preparation practices by using different ingredients, and avoiding salty, and spicy food. It was found that degree of favor for American food over Korean food was expanding with longer duration of resident in America. Also, the longer they live in America, the more American ingredients are used in food.

DISCUSSION

In the current epidemiological study using subjects of middle-aged men and women in Seoul, Korea and Columbus, OH, Koreans reported 6 gastric cancer incidence while the Korean-American immigrants incidence was one. The difference in cancer incidences between two groups was not statistically significant. The absence of statistical significance may be attributable to the small size of each group, which is 213 in Koreans and 244 Korean-Americans.

Comparison of dietary patterns showed that Korean-Americans and Koreans have different food consumption. Koreans preferred Kimchi, pickled fish, soybean
paste and rice while Korean-Americans preferred seaweed and brown rice. Even though Korean-Americans have been consuming substantial amounts of Korean food, their Korean food consumption was considerably lower than that of Koreans. Also, Korean-Americans’ Korean food was found to be adapted and modified to the new environment since they moved to America. Consequently, Korean-Americans consumed much less traditional Korean foods including Kimchi compared to their counterparts in Korea.

It has been reported that Kimchi improved digestion, prevented constipation, controlled intestinal flora, and had antimitogenic, anticarcinogenic, and other pharmaceutical functions (82). In addition, ascorbic acid, carotene, and dietary fiber in yellow-green vegetables, the extracts of red pepper, and the extracts of garlic used as Kimchi ingredients were believed to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicities induced by several carcinogens/mutagens (83).

However, there is much contradictory evidence. One case study reported that Kimchi intake increased the risk of stomach cancer whereas fresh vegetables were not associated with stomach cancer risk (14). It is suspected that Kimchi is highly associated with stomach cancer risk due to high salt, extreme spice and nitroso compounds.

High salt intake is known to induce atrophy in experimental animals (67) and to be associated with atrophic changes in the human gastric mucosa (84). Therefore,
there is high possibility that high salt intake would cause both gastritis and atrophy. Also, it was reported that intake of highly salted food may increase the mutagenicity of nitrosated foods (85). In a separate case control study, spicy and salty Korean foods such as soybean paste stew and hot pepper-soybean stew were found to be associated with stomach cancer risk (14).

There is evidence that extreme use of spices may increase the risk of stomach cancer. Although a mechanism was not identified, it was suspected that capsaicin, the hot-tasting component of chili peppers, may be carcinogenic as laboratory studies indicated. In Mexico, gastric cancer accounted for 10% of cancer death in 1990 (86). The high level of chili pepper consumption in Mexico may be a strong risk factor for gastric cancer (87). Animal studies also proved that capsaicin induced duodenal adenocarcinomas (88) and capsaicin was found to act as a promoter of stomach and liver tumors (89). Given that capsaicin is the main component of hot pepper, which is used in Kimchi manufacturing in Korea, it is highly suspected that consuming Kimchi would increase risk for stomach cancer.

Seel (90) reported that total nitrite load entering the stomach in their study averaged over 50 mg/day in Koreans due to high Kimchi intake, and that Americans consumed about 4.3 mg/day of nitrite. The high intake of nitroso compound was attributed to high nitrate contents in Kimchi and the quantity of this food consumed in the traditional diet, suggesting that Kimchi played an important role in the carcinogenesis of gastric cancer in Koreans.
Even though it is highly suspected that high Kimchi intake is closely related to the high gastric cancer incidence in Koreans, there are also many confounding factors which are associated in the current cohort study.

In the current study, socio-economic level of Korean-Americans was much higher than that of Korean respondents. That is, Korean-American respondents were found to have significantly higher education and more professional jobs than Korean respondents did (Table 3.1). Given that socio-economic level is inversely correlated with the incidence of stomach cancer (91), study subjects’ education and jobs could be a confounding factor. Also, Koreans were found to drink and smoke more than their counterparts in America (Table 3.2). It was found that alcohol consumption and tobacco smoking were correlated to stomach cancer risk (92). Another confounding factor could be the intake of antioxidants. Korean respondents were found to have a significantly lower intake of antioxidants than did Korean-American respondents (Table 3.3 & 3.4). Lower antioxidants intake might increase the stomach cancer risk in Koreans. In addition, Koreans were found to have consumed higher carbohydrates and lower proteins than did Korean-Americans (Table 3.3 & 3.4). Low-protein diets have been shown to inhibit the repair of gastric lesions by reducing gastric mucus production, thereby facilitating absorption of a potential gastric carcinogen. Also, high starch/low protein diets may reduce the concentration of agents in gastric juice that actively break down nitrites (93). The confounding factors mentioned previously might mask the effects of diet on gastric cancer risk in Koreans.
However, as in the current study, the importance of diet in gastric cancer risk has been commonly reported (94). Multiple epidemiology studies of differing designs have examined several aspects of Korean dietary practice and preferences in conjunction with stomach cancer (94). There is evidence that the choice of foods consumed, the taste preference and the methods of preparation influence risk. These results may reflect the effects of carcinogens or mutagens introduced to the diet. The fact that Korean-Americans had very different diets from Koreans (Table 3.5 and Figure 3.9) strongly suggested that the salty and spicy food such as Kimchi may play a role in increasing the stomach cancer risk in Koreans.
CHAPTER 4

MEASUREMENT OF GLUTATHIONE CONTENTS IN
SELECTED KOREAN FOODS
INTRODUCTION

Epidemiological studies on relation between diet and stomach cancer risk has mainly focused on the effects of fruits, dairy products, and meats, and a few components of foods such as dietary fiber, fats, and several vitamins (95). Although the results were not consistent always, a higher intake of fresh fruits and vegetetables was found to be connected with a lower risk of lung, stomach and colon cancer (96). Vitamin C, E, and other dietary antioxidants were suspected to be potential candidates as components which were responsible for these effects, although it was not confirmed (97). Glutathione, an antioxidant, was proven to be anticarcinogenic (98), can be biosynthesized in cells (99), has functions to prevent a cancer, conjugated xenobiotics to excrete as mercapturic acid (100), keeps functional form of vitamin C (101), involves DNA synthesis and repair (102), and enhances the immune response (103).

Glutathione, synthesized by γ-glutamyl glycine, is a ubiquitous and predominant nonprotein sulfhydryl compound in living organisms (33) and normally maintained at millimolar concentrations (104). It is a tripeptide, γ-glutamyl-L-cysteinyl-glycine, with an L-cysteinyl residue. Glutathione may be oxidized nonenzymatically and the oxidation may be involved with the activities of glutathione thiol transferase and of the glutathione peroxidases. The reaction is catalyzed by glutathione disulfide reductase and provides reducing power for the thiol transferases and peroxidases. In addition the reaction provides substrate for glutathione s-transferases. However, the synthetic
capacity of the human body is insufficient to maintain GSH level to function properly when organs are under exposure of xenobiotics such as drugs, redox cycling compounds, peroxides, X-rays, and ultraviolet radiation. Depletion or low level of GSH impairs the protection from these agents and produces injury or death in cells. Some epithelial cells, in addition to synthesis, also obtain glutathione from the blood by a sodium-dependent transport system (105). Therefore, gastrointestinal absorption of dietary glutathione may supplement glutathione synthesis in tissues to improve glutathione supply and glutathione-dependent detoxification. It was found that, hepatic glutathione content in laboratory animals varied as a function of dietary cysteine (or methionine, which is converted to cysteine in the liver) in amounts in the range of those required to maintain growth and nitrogen balance, indicating that dietary sulfur amino acid content may limit glutathione synthesis in vivo and result in altered glutathione-dependent detoxification (106). Thus, dietary sulfur amino acid content may affect tissue glutathione concentrations, and this in turn may be associated with protection against toxic or carcinogenic compounds.

When the stomach cancer mortality was compared worldwide, Korea was the leading country with 57.9 per 100,000 for males and 26.1 per 100,000 for females over the period of 1986-1987 (12). Since 1990, the results of 3 case-control studies (13-15) and one cohort study (16) on stomach cancer have been reported in Korea. Several food items were found to be associated with increased or decreased risk of stomach cancer among Koreans (13-15). High incidence of stomach cancer in Korea might be
associated with low intake of antioxidants, which might decrease the self-defense or self-immune systems in Koreans. However, there is no glutathione database for Korean food to investigate the glutathione intake in Korean. In Ohio, we analyzed the glutathione contents of selected Korean food.

METHOD AND MATERIAL

Chemicals

Yeast glutathione reductase EC1.6.4.2. NADPH 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), sodium phosphate (monobasic), EDTA, and glutathione were all purchased from Sigma Chemical Company (St. Louis, MO). The 5 % (w/v) perchloric acid used for extraction was prepared by dilution of 70 % perchloric acid with deionized water (107).

Assay procedure

Each individual food item was selected based on the most commonly reported items in the questionnaire survey both from Korean-Americans in Columbus and Koreans in Seoul. For each food item, at least three different brand name products or three different samples (different sources) were purchased locally from an oriental or Korean grocery store. Prior to sampling, non-edible portions were removed, foods were prepared according to most common cooking method in Korea. In general, steamed or boiled foods were cooked in a small amount of water until tender and then
drained. To determine the effects of cooking on the glutathione content, a few selected items were analyzed both in the raw and cooked state.

Food samples were homogenized (KinematicaCH-6010, Kriens-Lu) in a 5% perchloric acid solution (1:5 w/v) and centrifuged (Beckman Avanti-J25) at 10,000 x g for 5 minutes. The obtained supernatant was used in the assay. The reaction mixture consisted of 425 μL of DTNB, 50 μL of sample or standard, 425 μL of yeast glutathione reductase, and 100 μL NADPH. The amount of glutathione in the sample was determined by comparing the rate of absorption change to a standard curve (108).

RESULTS AND DISCUSSION

The glutathione content of selected Korean foods are reported in Table 4.1. The mean values of triplicate samples are provided with their standard deviation. The food items included in the current research were selected based on questionnaire responses.

The greatest glutathione content in Korean foods tested in the current study was found in dry kelp (55.0 ± 0.2 mg/100 g sample), followed by cooked laver (37.9 ± 2 mg/100 g sample), fresh sesame leaves (33.4 ± 0.1 mg/100 g sample), and anchovy (12.9 ± 0.03 mg/100 g sample). Differently, no glutathione was detected in noodle, pepper paste, pickled squid, cooked rice, soybean paste and yogurt. Small amounts of glutathione (< 9 mg glutathione/100 g sample) were also found in bean sprout, brown
rice, cucumber, egg plant, grain powder. Kimchi, kiwi, red pepper powder, uncooked rice, sesame leaves, soy milk, cooked sweet rice and tofu.
<table>
<thead>
<tr>
<th>Food</th>
<th>GSH (mg/100 g)</th>
<th>Food</th>
<th>GSH (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchovy (raw)</td>
<td>12.9 ± 0.03</td>
<td>Pepper paste</td>
<td>B/D*</td>
</tr>
<tr>
<td>Bean sprout (raw)</td>
<td>3.94 ± 0.1</td>
<td>Pickled squid</td>
<td>B/D*</td>
</tr>
<tr>
<td>Bean sprout (cooked)</td>
<td>2.33 ± 0.01</td>
<td>Red pepper powder</td>
<td>6.98 ± 1.2</td>
</tr>
<tr>
<td>Brown Rice (raw)</td>
<td>8.82 ± 0.17</td>
<td>Rice (raw)</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>Brown Rice (cooked)</td>
<td>1.78 ± 0.75</td>
<td>Rice (cooked)</td>
<td>B/D*</td>
</tr>
<tr>
<td>Cucumber</td>
<td>3.0 ± 1.0</td>
<td>Sesame leaf (pickled)</td>
<td>0.491 ± 0.2</td>
</tr>
<tr>
<td>Egg plant</td>
<td>0.53 ± 0.2</td>
<td>Sesame leaf (raw)</td>
<td>33.37 ± 0.1</td>
</tr>
<tr>
<td>Grain powder</td>
<td>4.71 ± 0.2</td>
<td>Soybean paste</td>
<td>B/D*</td>
</tr>
<tr>
<td>Kelp (dry)</td>
<td>55.0 ± 0.2</td>
<td>Soy milk</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Kimchi</td>
<td>0.45 ± 0.01</td>
<td>Sweet rice (cooked)</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Kiwi</td>
<td>2.23 ± 0.02</td>
<td>Tofu</td>
<td>1.79 ± 0.03</td>
</tr>
<tr>
<td>Laver (cooked)</td>
<td>37.9 ± 2</td>
<td>Yogurt</td>
<td>B/D*</td>
</tr>
<tr>
<td>Noodle</td>
<td>B/D*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Below detection limit

Table 4.1: Glutathione content in selected Korean foods
The glutathione content for cucumber measured in the current study (3.0 mg/100 g sample) was comparable to that reported in Jones et al. (109) (3.6 mg/100 g sample) using the HPLC method. However, the glutathione content for rice is considerably different between two studies (109). In the Jones study, they detected 1.6 ± 0.1 mg glutathione/100 g rice, while the current study found no glutathione in rice. This discrepancy may be attributable to the difference in cooking, processing and storage methods. Unlike cucumbers, rice is stored for long periods of time and cooked before serving, therefore, many factors can be associated with variation in glutathione content. In the current study, glutathione content in brown rice and white rice were significantly different, which clearly shows that processing methods contribute substantially to the glutathione content in rice. Further comparison was not possible due to lack of available data.

To determine the effect of food processing on the glutathione content, the glutathione content for bean sprouts, brown rice, white rice, and sesame leaves were measured in raw food and processed food. The processing methods used for bean sprouts, brown rice and white rice was cooking in small amount of water, and pickling (soaking in soy sauce with seasoning for a while) for the sesame leaves. In all cases, glutathione content decreased significantly in the processed foods, which showed that extreme caution should be given when the glutathione content for the processed food from different reports were compared.
A significant difference in glutathione content was found between Korean-Americans' and Koreans' favorite foods. Korean-Americans' favorite foods including brown rice, kelp, and laver had significantly higher glutathione content than Koreans' favorites such as white rice, noodle, pickled squid, soybean paste, egg plant and cooked bean sprout. Assuming that each group consumed the same amount of food, one could conclude that Korean-Americans consumed substantially higher amounts of glutathione than Koreans did.

Multiple epidemiological studies of differing designs have examined several aspects of Korean dietary practice and preferences in conjunction with stomach cancer (94). Multiple associations have been observed, but no single underlying factor has been identified to explain the high risk stomach cancer in this population. There is evidence that the choice of foods consumed, the taste preferences of the population, and the methods of preparation influence risk. These results may reflect the effect of carcinogens or mutagens introduced to the diet by such practices as pickling or broiling, reduction or protection resulting from low intake of fresh fruits and vegetables, or the combination of both increased exposure to carcinogens and reduced exposure to protective factors. The values reported in the present study do not provide a systematic assessment of the variations in GSH that could occur in foods as a consequence of seasonal, regional, varietal, and storage differences. However, this study can provide an experimental basis for assigning glutathione values for the items listed in the previous survey study.
CHAPTER 5

ANTIOXIDANT ENZYME ACTIVITY IN THE STOMACH AND OTHER DIGESTIVE ORGANS RELATED TO KIMCHI INTAKE
INTRODUCTION

Recent research has indicated that reactive oxygen-derived substances (RODS) are involved in the pathogenesis of several types of human diseases (110, 111), including conditions involving the gastrointestinal tract. The gastrointestinal epithelium is continuously exposed to RODS that can arise from luminal contents, such as ingested foods and drugs, sloughed mucosal cells and microorganisms, and also from the large number of inflammatory cells present in the gastrointestinal tract (112). Surface epithelial cells could present the first line of defense against such oxidants, provided that they were supplied with efficient antioxidant systems. The gastrointestinal mucosa is exposed to a variety of potentially noxious xenobiotics, including drugs, food constituents and food additives, which by interacting with cellular macromolecules, directly or following metabolic activation, may interfere with vital cell functions. Glutathione and the GSH-dependent enzyme systems provide major protection against such toxic agents (113).

Recent studies have established that some antioxidants, such as SOD, vitamin E, allopurinol, zinc, copper and selenium, are effective in the treatment or prevention of gastric mucosal injury (114), ischemic intestinal injury, colon cancer, and inflammatory bowel disease in animal models and human beings (115).

Diet appears to be involved in the early stages of transformation of normal cells into cancerous cells, with some factors acting as promoters and others as inhibitors. The initial stages of chronic gastritis and atrophy may be promoted by both
excessive salt intakes and infection with *Helicobacter pylori*. The intermediate stages of metaplasia and dysplasia are positively associated with the ingestion of nitrates, nitrites, or other factors that foster intragastric nitrosation, a process that may produce potent carcinogens (116).

Stomach cancer is prevalent all over the world, inter-country and intra-country differences in incidence exist, as well as different mortality rates between first generation immigrants and second generation immigrants. The stomach is where food first comes into prolonged contact with the gastrointestinal mucosa and the human diet contains a variety of both carcinogens and carcinogen antagonists. From these findings, it is reasonable to assume that dietary factors have the most important etiologic implications for stomach cancer (5).

Stomach cancer is the most common malignant neoplasm among Koreans and the age-standardized annual incidence of stomach cancer is among the highest in the world 57.9 per 100,000 for males and 26.1 per 100,000 for females over the period 1986-1987 (12). According to a Korea national nutrition survey, an adult consumes 50-100g of Kimchi/day in summer and 150-200g/day in winter, which constitutes 12.5% of total daily food intake (64). One of the case-control studies suggested that salted vegetables (such as Kimchi) may be associated with stomach cancer risk in Korea. Kimchi, having been consumed since the 3rd or 4th century in Korea (60), is a traditional, fermented Korean food that is prepared through a series of processes, including: pretreatment of oriental cabbage, brine, blending with various spices and
other ingredients- such as green onion, garlic, red pepper powder, shrimp sauce, and ginger-, and then fermented. Kimchi has been a major source of vitamins, minerals, and dietary fiber for Koreans especially during the winter (117).

However, little is known about the enzymatic and non-enzymatic antioxidant components of gastrointestinal mucosa in the stomach, small intestine, and colon related to Kimchi intake. The lack of information with regard to gastrointestinal mucosal antioxidant components along with the implication of RODS in a variety of gastrointestinal disorders led us to investigate the effects of Kimchi on the activities of the ubiquitous antioxidant enzyme; such as glutathione reductase, glutathione peroxidase, and glutathione S-transferase; and the levels of the non-enzymatic antioxidant glutathione in the mucosa of various gastrointestinal tract; namely, the body of the stomach, small intestine, colon and liver.

METHOD AND MATERIALS

Animals and Diets

Treatment of animals

Four weeks old male Wistar rats (75 ± 5g), purchased from Harlan, were housed individually in suspended stainless steel mesh cages. The animal room was kept at 21 °C and 40 % relative humidity with light from 0600 to 1800 h.

The rats were randomly assigned to one of four dietary treatment groups. Dyets (Bethlehem, PA) prepared the modified 76-AIN diets. The composition of diets
Table 5.1 shows. Kimchi was lyophilized (48 hours) using a freeze dryer (VirTris 800 SL). Kimchi replaced 5% or 10% cellulose portion of modified 76-AIN diet. All groups were fed a basal diet for 7 days. After this acclimatization, the animals were fed one of the four experimental diets. Sixty animals were divided into four diet groups of fifteen animals each and fed 5% cellulose, 5% Kimchi, 10% cellulose, and 10% Kimchi for 0, 2, and 4 weeks. The animals in each group (n=5) were allowed free access to food and water. Food was replenished with a freshly prepared diets every other day. Food consumption and gain in body weight were recorded every other day. After 0, 2, and 4 weeks the animals were sacrificed by carbon dioxide inhalation. Stomach, small intestine, colon, and liver were removed, immediately frozen in liquid nitrogen, and stored at -80 °C until analysis.
<table>
<thead>
<tr>
<th>ingredient</th>
<th>5% cellulose</th>
<th>5% Kimchi</th>
<th>10% cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>corn starch</td>
<td>55</td>
<td>55</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Kimchi</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>cellulose</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>corn oil</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>mineral mix</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 5.1: Comparison of rat diets (g/100 g)
Chemical reagents

Kimchi was purchased from local Korean grocery stores. Phosphate, EDTA, DTNB, GSH, GSSG, NADPH, Glutathione reductase (Type III from yeast), trichloroacetic acid, KCl, 1-chloro-2, 4-dinitrobenzene (CDNB), Dimethyl sulfoxide(DMSO), Hepes, t-butyl hydroperoxide, Glutathione disulfide, H\textsubscript{2}O\textsubscript{2}, triton, and Ethanol were purchased from Sigma Chemical (St. Louis, MO).

Tissue preparation

Liver, stomach, small intestine and colon (0.5g) were homogenized in 8-ml ice-cold 0.01M phosphate, 0.15 M KCl buffer at pH 7.4, for 10 seconds with Kinematica CH-6010 Kriens-Lu. Homogenized samples were centrifuged at 100,000 x g for 1 hr at 4 °C with a centrifuge (Beckman L7-65). The supernatant fraction was used as the source of the GSH S-transferase (EC 2.5.1.18), GSH peroxidase (EC 1.11.1.9), and GSSG reductase (EC 1.6.4.2). Protein concentration was assayed in triplicate by the method of Bradford (118) using bovine serum albumin as the standard with a spectrophotometer (Spectra Max 250, Beckman).

Assay for glutathione

Liver (0.5g), stomach (0.5g), small intestine (0.5g), and colon (0.5g) were homogenized (Kinematica CH-6010, Kriens-Lu) in 3-ml of 5% trichloroacetic acid for 10-seconds, then centrifuged (Beckman Avanti-J25) at 10,000 x g for 5-min at 4 °C.
The obtained supernatant fraction was analyzed for total GSH by the enzymatic method of Tietze (108). Each supernatant was diluted by 1/4, and the reaction mixture consisted of 80 μl of DTNB, 10 μl sample or standard, 80 μl glutathione reductase, and 30 μl of NADPH. The formation of conjugates of GSH with 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) was measured at 412 nm wavelength. The amount of glutathione in the sample was determined by comparing the rate of absorption change to a standard curve.

*Assay for GSH-Px Activity*

GSH-Px activities were assayed as described by Paglia and Valentine (119), and Prohaska and Gutsch (120) with slight modification. Reaction mixtures (200 μl) consisted of 40 μl GSH, 40 μl GSH reductase, 40 μl NADPH, 66 μl Hepes buffer and 10 μl of sample and was incubated at 37 °C for 3 min. Four μl -t-butyl hydroperoxide was added to begin the reaction. The decrease in absorbance of oxidized NADPH was monitored at 340 nm for 4 min. GSH-Px activities were determined by measuring absorbance and expressed as micromoles of NADPH oxidized per minute per gram of tissue.

*Assay for GSSG-Reductase activity*

GSSG-Reductase activities were assayed as described by Carlberg and Mannervik (56). The reaction mixtures (200 μl) contained 180 μl GSSG and NADPH mixture
and 20μl supernatant fraction from sample. The decreases in absorbance of NADPH as it becomes oxidized were monitored at 340 nm over 5 min. The extinction coefficient of NADPH, 6.3 X 10² l/mol mm, was used to calculate the activity of the reductase.

Assay for GST Activity

GST activities were assayed as described by Habig et al. (121) with slight modification. Briefly, the reaction mixture (400 μl) consisting of 320μl potassium phosphate (pH 7.0), 25 μl GSH, 25 μl sample, 25 μl TCA and 5 μl CDNB was incubated at 25 °C for 5 min. The reaction was initiated by the addition of CDNB, and absorbance was measured kinetically at 340 nm. The molar extinction coefficient for 1-chloro-2,4-dinitrophenylglutathione(CDNB) is 9.6 mM⁻¹cm⁻¹. Final results were expressed as micromoles of 1-chloro-2,4-dinitrophenylglutathione formed per minute per gram of tissue.

Statistical analysis

The statistical significance of the amount of Kimchi and time differences in glutathione or glutathione related enzymes in each tissues was assessed using two-way ANOVA at a significance level of 0.05, followed by the application of the Tukey test to assess the significance of specific intergroup differences using SigmaStat® 2.0 (SPSS Inc. Chicago, IL).
RESULTS

Table 5.2 shows the mean value of weight gain with standard deviation and mean value of food intake with standard deviation and feeding efficiency of rats. Feeding efficiency was obtained by dividing weight gain by feeding intake. Over a period of 2 weeks, rats gained from 163 g to 180 g, and ate from 273.8 g in the 5 % cellulose groups to 473.9 g in the 10 % Kimchi group. The feeding efficiency ranged from 0.58 in the 5 % cellulose group to 0.31 in the 10 % Kimchi group. Through all 4 groups, no statistical difference was found in weight gain; however, there was a significant difference in feeding intake between diet groups. That is, the 5 % cellulose group fed significantly less than the 10 % Kimchi group. Therefore, feeding efficiency was highest in the 5 % cellulose group, followed by the 5 % Kimchi, the 10 % cellulose, and the 10 % Kimchi groups.

<table>
<thead>
<tr>
<th>Different Diet</th>
<th>Weight gain (g/2 weeks)</th>
<th>Feed Intake (g/2 weeks)</th>
<th>Feeding Efficiency (weight gain/feed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 % Cellulose</td>
<td>180.6 ± 10α</td>
<td>273.8 ± 80α</td>
<td>0.58</td>
</tr>
<tr>
<td>5 % Kimchi</td>
<td>171.2 ± 9.5α</td>
<td>320.6 ± 97α</td>
<td>0.47</td>
</tr>
<tr>
<td>10 % Cellulose</td>
<td>176.9 ± 8.5α</td>
<td>360.3 ± 111α</td>
<td>0.45</td>
</tr>
<tr>
<td>10 % Kimchi</td>
<td>163.5 ± 9.8α</td>
<td>473.9 ± 145β</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 5.2: Effects of dietary Kimchi intake on feed intake and weight gain of rats (2 weeks). Values are mean for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey's test.
Table 5.3 shows the mean value of weight gain with standard deviation, mean value of food intake with standard deviation, and feeding efficiency in 4 weeks. Over 4 weeks period, rats gained from 310 g to 345 g, whereas their food consumption ranged from 642.8 g to 997.2 g. The feeding efficiency ranged from 0.54 in the 5 % cellulose group to 0.31 in the 10 % Kimchi group. As in 4-week results, there was no statistical significance in weight gain among groups, while statistical significance was found in food intake.

<table>
<thead>
<tr>
<th>Different Diet</th>
<th>Weight gain (g/4 weeks)</th>
<th>Feed Intake (g/4 weeks)</th>
<th>Feed Efficiency (weight gain/feed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 % Cellulose</td>
<td>345.1 ± 11.8*</td>
<td>642.8 ± 100*</td>
<td>0.54</td>
</tr>
<tr>
<td>5 % Kimchi</td>
<td>314.5 ± 25.1*</td>
<td>710.1 ± 100*ab</td>
<td>0.44</td>
</tr>
<tr>
<td>10 % Cellulose</td>
<td>327.4 ± 14.6*</td>
<td>937.2 ± 161bc</td>
<td>0.35</td>
</tr>
<tr>
<td>10 % Kimchi</td>
<td>310.0 ± 23.6*</td>
<td>997.2 ± 136c</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 5.3: Effect of dietary Kimchi intake on feed intake and weight gain of rats (4 weeks). Values are mean for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey's test
In Figure 5.1 the cumulative food intake for different diet rates is reported.

This figure shows that rats consumed more food in the order of 10% Kimchi group, 10% cellulose group, 5% Kimchi group, and 5% cellulose group.

Figure 5.2 shows the cumulative weight gain in different diet rats. Cumulative weight gain is displayed as a straight line.

Figure 5.1: Accumulative food intake in different diet rats
Figure 5.2: Accumulative weight gain in different diet rats

In Figure 5.3, the relationship between food intake and weight gain for four treatments is reported.

In Figure 5.4, the relationship between food intake and weight gain for the 5% cellulose and the 5% Kimchi group is reported.

In Figure 5.5, the relationship between food intake and weight gain for the 10% cellulose and the 10% Kimchi group is reported.
Figure 5.3: The relationship between food intake and weight gain for four treatment groups
Figure 5.4: The relationship between food intake and weight gain for 5% Cellulose and 5% Kimchi
Figure 5.5: The relationship between food intake and weight gain for 10% Cellulose and 10% Kimchi.
Table 5.4 shows the difference in GSH content in rat liver among different
groups over a 4 week test period. The glutathione content at week 0 was not
significantly different between groups; thus, the values were pooled and reported in
one mean value, which was 11.95 μmol/g tissue. The glutathione content in livers
increased as rats grew, ranging from 11.95 μmol/g tissue at week 0 to 21.52 μmol/g
tissue in 4 weeks. In 2 weeks, the glutathione content in the 10% Kimchi group was
significantly lower than those of other groups. In 4 weeks, glutathione content in the 5
% cellulose group was lowest (17.63 ± 2.0 μmol/g tissue), while that in the 10%
cellulose group was highest (21.52 ± 1.9 μmol/g tissue).

Figure 5.6 shows the difference in GSH content in the rat liver for four diet
treatment groups.

<table>
<thead>
<tr>
<th>GSH-Liver (μmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>11.95 ± 1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>16.23 ± 1.5ab</td>
<td>16.80 ± 1.1a</td>
<td>14.64 ± 1.3ab</td>
<td>14.24 ± 1.1b</td>
</tr>
<tr>
<td>4 week</td>
<td>17.63 ± 2.0*</td>
<td>20.49 ± 1.9ab</td>
<td>21.52 ± 1.9b</td>
<td>21.38 ± 0.7ab</td>
</tr>
</tbody>
</table>

Table 5.4: GSH contents in the rat liver. The means with same superscripts in a row
are not statistically different. Values are means for 5 animals per group.
Means within a column not sharing a common letter are significantly
different (p < 0.05) by Tukey’s test.
Figure 5.6: Glutathione contents in the liver
Table 5.5 shows the glutathione content in the rat stomach in 4 different groups over 4 weeks. Overall, glutathione content in the stomach also increased with increasing time. However, the difference was much smaller than that of glutathione content in the liver. In 2 weeks, glutathione content ranged from 6.45 μmol/g tissue in the 5% Kimchi group to 7.29 μmol/g tissue in the 10% Kimchi group. In 4 weeks, glutathione content ranged from 7.57 μmol/g tissue to 8.23 μmol/g tissue. However, there was no statistical difference found between groups. Figure 5.7 shows the GSH content in rat stomach for four treatment groups.

<table>
<thead>
<tr>
<th>GSH-stomach (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>2.88 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>7.12 ± 0.6a</td>
<td>6.45 ± 0.3b</td>
<td>7.05 ± 0.2a</td>
<td>7.29 ± 0.5a</td>
</tr>
<tr>
<td>4 week</td>
<td>7.57 ± 0.5a</td>
<td>8.23 ± 0.3a</td>
<td>7.83 ± 0.2a</td>
<td>7.75 ± 0.7a</td>
</tr>
</tbody>
</table>

Table 5.5: GSH contents in the rat stomach. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.7: Glutathione contents in the stomach
Glutathione content in the rat small intestine is reported in Table 5.6. At week 0, 3.38 µmol/g tissue of glutathione was detected in small intestine. The content increased with increasing time. In 2 weeks, glutathione contents were low in the 5% cellulose and the 5% Kimchi groups (4.13 ± 0.3 µmol/g tissue and 4.12 ± 0.4 µmol/g tissue, respectively), while those of the 10% cellulose and the 10% Kimchi groups (4.86 ± 0.1 and 5.18 ± 0.4 µmol/g tissue) were significantly higher than two other groups. In 4 weeks, glutathione contents were very similar between groups, ranging from 5.29 to 5.81 µmol/g tissue. Figure 5.8 shows GSH in the small intestine.

<table>
<thead>
<tr>
<th>GSH-Small intestine (µmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>3.38 ± 0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>4.13 ± 0.3</td>
<td>4.12 ± 0.4</td>
<td>4.86 ± 0.1</td>
<td>5.18 ± 0.4</td>
</tr>
<tr>
<td>4 week</td>
<td>5.68 ± 0.4</td>
<td>5.89 ± 0.3</td>
<td>5.29 ± 0.4</td>
<td>5.81 ± 0.3</td>
</tr>
</tbody>
</table>

Table 5.6: GSH contents in the rat small intestine. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey's test.
Figure 5.8: Glutathione content in the small intestine
Table 5.7 shows the glutathione content in the rat colon between groups over a 4 week period. At week 0, 2.45 ± 0.3 μmol/g tissue of glutathione was found in the rat colon. After that, glutathione content in colon increased with increasing time. In 2 weeks, the greatest amount of glutathione was found in the 10% cellulose groups (3.49 ± 0.3 μmol/g tissue), followed by the 10% Kimchi, 5% Kimchi, and 5% cellulose group. The 10% Kimchi group had significantly higher glutathione content than other groups. In 4 weeks, the content increased in the order of the 10% Kimchi (3.77 ± 0.1 μmol/g tissue), 10% cellulose (3.49 ± 0.1 μmol/g tissue), 5% Kimchi (tissue 3.09 ± 0.2 μmol/g tissue), and 5% cellulose groups (2.79 ± 0.1 μmol/g tissue) and was significantly different between all 4 groups. Figure 5.9 shows the GSH content in the rat colon for four treatment groups.

<table>
<thead>
<tr>
<th>GSH-Colon (μmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>2.45 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>2.54 ± 0.1a</td>
<td>2.64 ± 0.2a</td>
<td>3.49 ± 0.1b</td>
<td>3.21 ± 0.3b</td>
</tr>
<tr>
<td>4 week</td>
<td>2.79 ± 0.1a</td>
<td>3.09 ± 0.2b</td>
<td>2.94 ± 0.1ab</td>
<td>3.77 ± 0.1c</td>
</tr>
</tbody>
</table>

Table 5.7: GSH contents in the rat colon. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.9: Glutathione content in the colon
Glutathione peroxidase (GPx) content in liver is reported in Table 5.8. At week 0, 80.02 ± 2.5 μmol/g tissue of GPx was detected in rat liver. Also, GPx content increased with increasing time. In 2 weeks, a relatively high content of GPx was detected in the 5% cellulose (103.21 ± 3.5 μmol/g tissue) and 10% cellulose groups (99.91 ± 2.3 μmol/g tissue); while relatively low content of GPx was detected in the 5% Kimchi (84.43 ± 3.5 μmol/g tissue) and 10% Kimchi groups (83.47 ± 5.6 μmol/g tissue). In 4 weeks, significantly higher GPx content was found in 5% cellulose (154.85 ± 5.0 μmol/g tissue) and 10% Kimchi groups (157.26 ± 12.8 μmol/g tissue), while relatively low GPx concentration was present in 5% Kimchi (135.00 ± 3.3 μmol/g tissue) and 10% cellulose groups (135.09 ± 7.1 μmol/g tissue). Figure 5.10 demonstrated glutathione peroxidase activity in the liver.

<table>
<thead>
<tr>
<th>LGPx (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>80.02 ± 2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>103.21 ± 3.5</td>
<td>84.43 ± 3.5</td>
<td>99.91 ± 2.3</td>
<td>83.47 ± 5.6</td>
</tr>
<tr>
<td>4 week</td>
<td>154.85 ± 5.0</td>
<td>135.00 ± 3.3</td>
<td>135.09 ± 7.1</td>
<td>157.26 ± 12.8</td>
</tr>
</tbody>
</table>

Table 5.8: GPx activity in the rat liver. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.10: Glutathione Peroxidase activity in the liver.
Table 5.9 presents the data on GPx in the stomach. GPx at week 0 was 7.51 ± 0.6 μmol/g tissue. As usual, GPx content in the stomach increased with increasing time. In 2 weeks, GPx content in stomach ranged from 7.55 to 13.14 μmol/g tissue, with the lowest in the 10% Kimchi group (7.55 ± 0.5 μmol/g tissue) and the highest in the 5% cellulose group (13.14 ± 0.9 μmol/g tissue). In 4 weeks, significantly higher GPx contents were found in the 5% cellulose and 5% Kimchi groups, while significantly lower GPx contents were found in the 10% cellulose and 10% Kimchi groups. Figure 5.11 presents the data on glutathione peroxidase activity in the rat stomach.

<table>
<thead>
<tr>
<th>SGPX (µmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>7.51 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>13.14 ± 0.9a</td>
<td>9.63 ± 1.0b</td>
<td>10.88 ± 1.1b</td>
<td>7.55 ± 0.5c</td>
</tr>
<tr>
<td>4 week</td>
<td>14.82 ± 0.6a</td>
<td>13.79 ± 0.7a</td>
<td>11.14 ± 0.4b</td>
<td>10.14 ± 0.8b</td>
</tr>
</tbody>
</table>

Table 5.9: GPx activity in the rat stomach. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) according to Tukey’s test.
Figure 5.11: Glutathione Peroxidase activity in the stomach
GPx content in the small intestine is presented in Table 5.10. At the beginning, 6.95 ± 1.3 μmol/g tissue GPx was present in the small intestine, which increased with growth of rats. In 2 weeks, the GPx content in the rat small intestine ranged from 9.39 ± 1.8 in the 10% Kimchi group to 13.05 ± 0.9 μmol/g tissue in the 5% cellulose group. In 4 weeks, the same trend was found with a range of 10.03 to 14.07 μmol/g tissue. Figure 5.12 presents the data on glutathione peroxidase in the rat small intestine.

<table>
<thead>
<tr>
<th>Small Intestine GPx (μmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>6.95 ± 1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>13.05 ± 0.9^a</td>
<td>11.13 ± 2.6^{ab}</td>
<td>9.9 ± 1.1^b</td>
<td>9.39 ± 1.8^b</td>
</tr>
<tr>
<td>4 week</td>
<td>14.07 ± 1.9^a</td>
<td>12.96 ± 1.8^{ab}</td>
<td>10.03 ± 0.8^b</td>
<td>10.52 ± 1.2^b</td>
</tr>
</tbody>
</table>

Table 5.10: GPx activity in the rat small intestine. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.12: Glutathione Peroxidase in the small intestine
In Table 5.11, GPx content in the colon is reported. At week 0, 1.08 ± 0.7 μmol/g tissue of GPx was present in the colon. In 2 weeks, GPx content in colon ranged from 0.38 (10% Kimchi group), which was significantly lower than others, to 2.31 μmol/g tissue (5% cellulose group). In 4 weeks, GPx ranged from 1.67 in 10% Kimchi to 4.39 μmol/g tissue in 5% cellulose, which was significantly higher than others. Figure 5.13 demonstrated glutathione peroxidase activity in the colon.

<table>
<thead>
<tr>
<th>Colon GPx (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>1.08 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>2.31 ± 0.4a</td>
<td>1.15 ± 0.4b</td>
<td>1.85 ± 0.5a</td>
<td>0.38 ± 0.1c</td>
</tr>
<tr>
<td>4 week</td>
<td>4.39 ± 0.3a</td>
<td>2.03 ± 0.3bc</td>
<td>2.84 ± 0.7b</td>
<td>1.67 ± 0.5c</td>
</tr>
</tbody>
</table>

Table 5.11: GPx activity in the rat colon. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.13: Glutathione Peroxidase activity in the colon
Table 5.12 shows the glutathione reductase activity in the rat liver. It did not show significant difference among groups. At week 0, 14.17 µmol/g tissue glutathione reductase was detected in the rat liver. In 2 weeks, the glutathione reductase content increased from that of week 0, however, no significant was found between groups with the highest content in the 5% cellulose group (16.35 ± 2.6 µmol/g tissue) and with the lowest content in the 10% Kimchi group (14.00 ± 2.2 µmol/g tissue). In 4 weeks, glutathione reductase activity ranged from 14.24 ± 1.3 to 21.54 ± 2.5 µmol/g tissue which was significantly different from the rest of the groups. Figure 5.14 reports the glutathione reductase activity in the liver.

<table>
<thead>
<tr>
<th>Liver-GR (µmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>14.17 ± 1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>16.35 ± 2.6a</td>
<td>14.67 ± 2.1a</td>
<td>16.35 ± 1.9a</td>
<td>14.00 ± 2.2a</td>
</tr>
<tr>
<td>4 week</td>
<td>21.54 ± 2.5a</td>
<td>14.92 ± 1.9b</td>
<td>17.11 ± 2.1b</td>
<td>14.24 ± 1.3b</td>
</tr>
</tbody>
</table>

Table 5.12: Glutathione reductase activity in the rat liver. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey's test.
Figure 5.14: Glutathione Reductase activity in the liver
Table 5.13 shows the glutathione reductase activity in the rat stomach. The 0-week rats showed lowest activity which was $11.85 \, \mu\text{mol/g tissue}$. In 2 weeks, the activity increased from the week 0 except for the 10% Kimchi group. In 2 weeks, the activity ranged from 10.80 to 14.02 $\mu\text{mol/g tissue}$, but there was no statistical difference between groups. Even in 4 weeks, there was no significant difference between groups. Figure 5.15 presents the glutathione reductase activity in the rat stomach.

<table>
<thead>
<tr>
<th>Stomach-GR (µmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>11.85 ± 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>14.02 ± 1.8*</td>
<td>12.62 ± 0.8*</td>
<td>12.43 ± 1.6*</td>
<td>10.80 ± 2.4*</td>
</tr>
<tr>
<td>4 week</td>
<td>18.37 ± 1.6*</td>
<td>17.49 ± 1.5*</td>
<td>17.15 ± 0.6*</td>
<td>16.68 ± 0.4*</td>
</tr>
</tbody>
</table>

Table 5.13: Glutathione reductase in the rat stomach. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different ($p < 0.05$) by Tukey's test.
Figure 5.15: Glutathione Reductase activity in the stomach
Table 5.14 shows the glutathione reductase activity in the rat small intestine.

At week 0, rats had $8.45 \pm 1.0$ μmol glutathione reductase/g tissue. In 2 weeks, 10% Kimchi group had the lowest glutathione reductase content ($7.68 \pm 1.9$ μmol/g tissue), while 5% cellulose group had the highest $10.54 \pm 0.5$ μmol/g tissue. In 4 weeks, the content increased from that of 2 weeks with the range of 9.4 to 10.3 μmol/g tissue, but there was no significant difference between groups. Figure 5.16 shows the glutathione reductase activity in the small intestine.

<table>
<thead>
<tr>
<th>Small intestine GR (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>8.45 ±1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>10.54 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.93 ± 1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.02 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.68 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 week</td>
<td>10.03 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.29 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.69 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.40 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 5.14: Glutathione reductase in the rat small intestine. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different ($p < 0.05$) by Tukey's test.
Figure 5.16: Glutathione Reductase activity in the small intestine
Table 5.15 shows the glutathione reductase in the rat colon. At 0 week, glucathione reductase content in the colon was 7.92 μmol/g tissue. In 2 weeks, the content increased in the 5% and 10% cellulose groups, while it decreased in the 5% and 10% Kimchi groups. Thus, the glutathione content in Kimchi group was significantly lower than that of cellulose groups. In 4 weeks, the 5% cellulose group (13.25 ± 0.9) show highest enzyme activity among groups, followed by the 10% cellulose group (10.23 ± 1.4), 10% Kimchi (9.97 ± 0.1), and 5% Kimchi (7.74 ± 1.1) groups. In 4 weeks, the 5% Kimchi group has the least glutathione reductase activity, while the 5% cellulose group had the highest. Figure 5.17 shows the glutathione reductase activity in the colon.

<table>
<thead>
<tr>
<th>Colon GR (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>7.92 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>11.29 ± 1.0^a</td>
<td>7.20 ± 0.3^b</td>
<td>10.33 ± 0.8^a</td>
<td>7.05 ± 1.8^b</td>
</tr>
<tr>
<td>4 week</td>
<td>13.25 ± 0.9^a</td>
<td>7.74 ± 1.1^c</td>
<td>10.23 ± 1.4^b</td>
<td>9.97 ± 0.1^b</td>
</tr>
</tbody>
</table>

Table 5.15: Glutathione reductase activity in rat colon. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.17: Glutathione Reductase activity in the colon
Table 5.16 shows the glutathione-S-transferase (GST) activity in the rat liver. In 0 week, GST activity in the rat liver was $292.42 \pm 4.7$ µmol/min/g tissues. The 5% cellulose group showed highest increase in glutathione enzyme activity. The highest GST content was found in the 5% cellulose group ($353.90 \pm 5.6$), followed by the 10% cellulose group ($324.19 \pm 1.9$), 10% Kimchi group ($317.93 \pm 2.8$) and 5% Kimchi ($314.82 \pm 4.6$). At four weeks there was a significant difference between groups, with the highest in the 5% cellulose group $463.46 \pm 5.3$ µmol/g tissue. Figure 5.18 shows the glutathione-S-transferase activity in the rat liver.

<table>
<thead>
<tr>
<th>Liver-GST (µmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>292.42 ± 4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>353.90 ± 5.6a</td>
<td>314.82 ± 4.6b</td>
<td>324.19 ± 1.9c</td>
<td>317.93 ± 2.8bc</td>
</tr>
<tr>
<td>4 week</td>
<td>463.46 ± 5.3a</td>
<td>322.17 ± 4.1b</td>
<td>326.86 ± 3.8bc</td>
<td>332.56 ± 2.3c</td>
</tr>
</tbody>
</table>

Table 5.16: GST activity in the rat liver. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.18: Glutathione-S-Transferase activity in the liver
Table 5.17 shows the glutathione-S-transferase (GST) activity in the rat stomach. At week 0, 17.5 μmol GST/g tissue was determined. GST content ranged from 18.44 to 22.74 μmol/g tissue in 2 weeks without statistical differences. Even at 4 weeks, there was no significant difference in GST enzyme content, with a range from 24.02 to 25.62 μmol/g tissue. Figure 5.19 shows glutathione-s-transferase activity in the stomach.

<table>
<thead>
<tr>
<th>Stomach-GST (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>17.5 ±2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>22.74 ± 3.7a</td>
<td>22.46 ± 2.6a</td>
<td>21.17 ± 4.1a</td>
<td>18.44 ± 3.2a</td>
</tr>
<tr>
<td>4 week</td>
<td>25.62±3.1a</td>
<td>24.21±2.3a</td>
<td>24.02 ±1.4a</td>
<td>24.25 ± 4.3a</td>
</tr>
</tbody>
</table>

Table 5.17: GST activity in the rat stomach. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.19: Glutathione-S-Transferase activity in the stomach
Table 5.18 shows GST activity in the rat small intestine. At week 0, 9.37 μmol/g tissue of GST was detected. In 2 weeks, GST content in the small intestine was the highest in the 5% cellulose group (12.92 ± 3.1) followed by 5% Kimchi (11.7 ± 4.0), 10% cellulose (10.62 ± 6.5), and 10% Kimchi (8.67 ± 0.9) group; even though there was no significant difference among groups. Even at 4 weeks, the content was not significantly different between groups, ranging from 12.19 to 14.96 μmol/g tissue. Figure 5.20 shows the glutathione-s-transferase activity in the small intestine.

<table>
<thead>
<tr>
<th>Small intestine GST (μmol/g tissue)</th>
<th>5% Cellulose</th>
<th>5% Kimchi</th>
<th>10% Cellulose</th>
<th>10% Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>9.37 ±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 week</td>
<td>12.92 ± 3.1^a</td>
<td>11.7 ± 4.0^a</td>
<td>10.62 ± 6.5^a</td>
<td>8.67 ± 0.9^a</td>
</tr>
<tr>
<td>4 week</td>
<td>14.96 ±3.7^a</td>
<td>12.91 ± 3.1^a</td>
<td>13.65 ± 2.1^a</td>
<td>14.01 ± 2.8^a</td>
</tr>
</tbody>
</table>

Table 5.18: GST activity in the rat small intestine. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey’s test.
Figure 5.20: Glutathione-S-Transferase activity in the small intestine
Table 5.19 shows the GST activity in the rat colon. At week 0, GST content in the rat colon was 6.98 μmol/g tissue which increased with increasing time. In 2 weeks, the content ranged from 5.70 to 8.73 μmol/g tissue and ranged from 7.08 to 9.70 μmol/g tissue in 4 weeks. There were no significant differences found between groups in the GST content, even though it increased with time. Figure 5.21 shows the glutathione-s-transferase activity in the colon.

<table>
<thead>
<tr>
<th>Colon GST (μmol/g tissue)</th>
<th>5 % Cellulose</th>
<th>5 % Kimchi</th>
<th>10 % Cellulose</th>
<th>10 % Kimchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>6.98 ± 1.5</td>
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<tr>
<td>2 week</td>
<td>8.73 ± 3.8a</td>
<td>7.58 ± 1.3a</td>
<td>6.75 ± 1.2a</td>
<td>5.70 ± 1.8a</td>
</tr>
<tr>
<td>4 week</td>
<td>9.70 ± 2.2a</td>
<td>9.23 ± 2.4a</td>
<td>7.96 ± 0.9a</td>
<td>7.08 ± 1.4a</td>
</tr>
</tbody>
</table>

Table 5.19: GST activity in the rat colon. The means with same superscripts in a row are not statistically different. Values are means for 5 animals per group. Means within a column not sharing a common letter are significantly different (p < 0.05) by Tukey's test.
Figure 5.21: Glutathione-S-Transferase activity in the colon
DISCUSSION

The activities of the endogenous antioxidant components, glutathione reductase, glutathione peroxidase, glutathione-S-transferase, and glutathione in the stomach, small intestine, colon, and liver of male Wistar rats have been investigated to study diet dependent variations. These variations may in turn influence susceptibility to oxidant injury. The activity of glutathione peroxidase, measured in this study, was significantly affected by both the amount of Kimchi, from 0 week to 2 weeks time period.

The gastrointestinal mucosa is exposed to a variety of potential noxious xenobiotics, including drugs, food constituents, and food additives which by interacting with cellular macromolecules either directly or following metabolic activation, may interfere with vital cell functions. Glutathione and glutathione-related enzymes provide major protection against such toxic agents (113). High concentrations of glutathione were found in the stomachs of rodents (122) and humans (123). In general, the mucosa of the small intestine showed moderately higher levels of GSH as compared with those of the stomach or colon (124). The differences may be attributable to the fact that most of the glutathione present in the diet is bioavailable in the small intestine due to the presence of Na⁺-dependent and Na⁺-independent uptake mechanisms in the cells of small intestine. However, the current study showed that higher levels of GSH in the stomach compared with those of the small intestine and colon. The possibility that the high levels of glandular gastric GSH may be an
important modulatory factor in the induction of gastric cancer by highly reactive electrophilic chemicals or metabolites should be explored.

Glutathione is utilized in reduced form in numerous biological reactions, and glutathione reductase is of prime importance in the metabolic function of glutathione. Even though no statistically significant differences were found, glutathione reductase activity in the stomach was consistently lower in the 10% Kimchi groups both for 2- and 4- weeks. Therefore, biological differences are highly suspected to exist. Due to low activity of glutathione reductase in the stomach, the 10% Kimchi group might be subjected to the glandular stomach cancer more easily than other dietary treatment groups.

Glutathione peroxidase in the stomach showed the statistical differences between Kimchi and cellulose groups with the least enzyme activity in the 10% Kimchi group at 2 weeks, however, the 4 week data did not show the statistical differences between the 10% Kimchi group and the 10% cellulose group but showed the significant differences between 10% Kimchi group and 5% cellulose group. Besides the low glutathione peroxidase activity in the 10% Kimchi group, the enzyme activity in 10% cellulose group was also significantly lower from 5% Kimchi and 5% cellulose groups, which might be associated with lower energy intake due to high content of fiber, since the rats were in rapidly growing stages of life. With increasing time from 2 to 4 weeks, the difference between groups decreased, which can be attributable to adaptation mechanisms.
The higher amount of GSH in the colon points to the importance of GSH in protecting against noxious compounds entering the body via the gastrointestinal tract in 10 % Kimchi group. Total GSH concentration, as well as the ability to maintain GSH in a reduced state via glutathione reductase, are important antioxidant defenses. Over time, GSH concentration in 4 weeks was high in the colon of 10 % Kimchi group, but their glutathione reductase activity was low, therefore, reduced glutathione content in colon is required to see the exact amount of glutathione.

Low GST activities in the stomach, small intestine, colon in 2 weeks have been purported to account for a higher susceptibility of those tissues to carcinogenesis (125). In spite of the absence of overt oxidative damage, it is of interest to determine to what extent the antioxidant capabilities of stomach, small intestine, and colon mucosa have been compromised from diet alone. Initiators of radical formation in tissues are abundant in our environment: they are part of current lifestyle practices and medical care (126). The fact that GST activity of the 10 % Kimchi group in the small intestine and stomach was low, even though no statistical significance existed, at 2 weeks and that the activity substantially increased in 4 weeks indicated that the tissues had the ability to offset the additional stress from Kimchi intake. In conclusion, the present study demonstrated that antioxidant mechanisms in the stomach, small intestine, and colon mucosa of rats were affected by the Kimchi diet.

The hypothesis that high intake of Kimchi would affect the antioxidant enzyme activity in the stomach could not be supported from these data alone. Implication
from the observed changes in glutathione related enzymes are facts that rats can produce vitamin C, which cannot be produced in humans. It was found that most enzyme activity increased from 0 weeks to 4 weeks in which their real age was 2 months old. It might be associated with the fact that rats were in the stage of rapid growth. It was also reported that antioxidant enzyme activities of rats increased up to 4 months of age and decreased with age (127). Therefore, the older rats may be better animal models for determining the effects of Kimchi. In summary, the present study demonstrated that antioxidant mechanisms in the stomach, small intestine, and colon were affected by the amount of Kimchi and by the feeding period. Even though they did not show a significant difference among groups, the 10% Kimchi group had the least enzyme activity in all enzymes in the stomach, small intestine, colon and liver. AIN-76 diet contains adequate vitamins and minerals that are classified as nonenzymatic antioxidants and the presence of these antioxidants may attenuate the effect of dietary Kimchi. Also, our experimental periods were too short to see the exact mechanism. That is, the rats were in a growing period, so, most enzyme activity increased with age in the organs which might mask the effect of the diet on enzyme activity.
CHAPTER 6

SUMMARY

Stomach cancer is the most common cancer in Korea. However, stomach cancer mortality among Korean-Americans is much lower than that of Koreans, even though the incidence in Korean-Americans is still significantly higher than that of Americans. Given that Koreans and Korean-Americans are sharing a similar genetic background, it is highly suspected that the environmental factors including diets are contributing to the difference in stomach cancer incidences between Koreans and Korean-Americans. To determine the effects of dietary factors on the stomach cancer incidence of Koreans, an epidemiology study was performed. Three hundred questionnaires consisted of demographic information and 2-d weighed dietary records were distributed each in Seoul, Korea and Columbus, OH. From the returned questionnaires, it was revealed that Koreans and Korean-Americans had very distinctive dietary patterns. Six cancer incidences were recognized among 213 Koreans, while only one incidence was reported among 244 Korean-Americans. Also, Koreans were found to consume more white rice, Kimchi, soybean paste stew, red pepper paste and pickled fish compared to their counterparts in America, while
Korean-Americans were discovered to eat more seaweed, kelp, laver, and raw vegetables. In other words, Korean-Americans’ diet contained significantly less salt or spice compared to Koreans’. In addition, Koreans’ diets were found to be very monotonous, while Korean-Americans’ were varied in terms of micronutrient source. Koreans were found to consume high carbohydrate and low protein diet, while Korean-Americans had low carbohydrate and high protein diets. For protein sources, Koreans ingested proteins mainly from vegetables, while Korean-Americans consumed proteins primarily from animal sources.

To determine the difference in glutathione consumption between Koreans and Korean-Americans, the glutathione contents of foods that were frequently reported in returned questionnaires were measured. The Korean-Americans’ favorite foods including kelp, laver, and brown rice were found to contain high levels of glutathione, while Koreans’ favorite foods including white rice, Kimchi, pickled sesame leaf, pickled fish, and pepper paste carried glutathione below the detection limit. Accordingly, Korean-Americans were found to consume foods with considerably higher glutathione content than the Koreans did.

Based on the questionnaire respondents that Koreans consumed significantly higher Kimchi than their counterparts and the fact that Kimchi contains very high amounts of salts and spices, animal models were used to determine the effects of Kimchi on the antioxidant defense enzyme system in the digestive system. Four week old Wistar rats were fed AIN 76 modified diet with different portion of Kimchi and
cellulose in the time interval of 0, 2, and 4 weeks. Interestingly, the rats in Kimchi groups gained less weight than those in cellulose groups, regardless that the rats in Kimchi groups feed substantially more than those in cellulose groups. Consequently, feeding efficiency in Kimchi groups were lower than that in cellulose groups. Enzyme activities increased with increasing time because the rats were growing rapidly. Glutathione peroxidase activities were lower in 10% Kimchi groups than 5% cellulose groups for all three digestive organs including stomach, small intestine, and colon. Glutathione reductase activities in Kimchi groups was not significantly different in the stomach and the small intestine even though the activities were lower than cellulose groups, however, the glutathione reductase activities in Kimchi groups were significantly lower than those in cellulose groups in the colon. Glutathione transferase activities in Kimchi groups were lower than those in cellulose groups, but no significant differences were found. Glutathione content in the colon was significantly higher in Kimchi groups, but the content was not significantly different between groups in other digestive organs such as small intestine and stomach.

In the epidemiological study, Koreans were found to have higher stomach cancer incidence and consumed more Kimchi than Korean-Americans did, therefore, the correlation between Kimchi consumption and stomach cancer incidence was suspected. However, the significant correlation between Kimchi consumption and antioxidant enzyme activity was not conspicuous always in the animal models. This may be attributable to the short exposure duration and the use of too young animals.
BIBLIOGRAPHY


Appendix: A

SURVEY QUESTIONNAIRE
Questionnaire

Human Nutrition and
Food Management
Campbell, 1787 Neil Avenue
Columbus, OH 43210
Tel) 614-292-4485

Jun. 10, 1996
Dear Mr. Or Mrs.

The Ohio State University is conducting a study on the dietary preferences of Korean immigrants. Your names were obtained from the Korean Society Association of Columbus and referrals from current members. This is an opportunity for you to make a difference in nutritional research. There is great need for research involving the Korean population and their eating habits.

By having the husband or wife of your household complete the enclosed questionnaire, you can provide important information about Korean food choices related to stomach disease. Your responses will help us compare how frequently certain foods are consumed by Koreans in the United States relative to those in Korea. We are interested in your food choices made after immigration to the United States from Korea. If the husband or wife minimum of 40 years of age is ask that you complete the enclosed questionnaires. We would like you to return before June 30.

Self-addressed stamped envelopes are enclosed for the questionnaires. Names and responses will be kept confidential. Please be sure to fill out the appropriate questionnaire.

If you have any questions please call Kyeok Kim at (614) 297-6765 or Dr. Bray at (614) 292-4485.

Sincerely,

Tammy. M. Bray, Ph. D. Kyeok Kim
Professor of Human Nutrition & Food Management Graduate Student

Enclosure:
QUESTIONNAIRE

Pleases circle the appropriate letter or fill in the blank with the appropriate answer.

1. What is your gender?
   A: male
   b: female

2. What is your age in years? __________

3. Where do you live (please circle your answer): Columbus city - more than 100,000
   a. large city (over 100,000)
   b. medium city (50,000-100,000)
   c. small city (10,000-49,000)
   d. town (2,500-9,999)

4. What's your weight and height?
   a. wt : __________
   b. ht : __________

5. Please circle the highest grade in school you have completed:
   1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16 +

6. How long (in years) have you lived in the United States? _________

7. What is/was your occupation when employed or before retirement?

8. Are you on a special diet?
   a. no
   b. yes

* if yes, please, encircle the relevant item below:
   a. weight loss
   b. diabetic diet
   c. vegetarian
   d. low salt
   e. low cholesterol/fat
   f. weight gain
   g. other
9. What kinds of health problems do you have?
   a. none
   b. heart condition
   c. obesity
   d. high blood pressure
   e. diabetes
   f. cancer – liver, lung, stomach, other
   g. other

10. Do you have a relatives with a stomach cancer?
    a. yes
    b. no

* What do you think is the main cause of the cancer?
  a. Kimchi
  b. salted food
  c. smoked meat (i.e. burned meat)

11. Have you taken any medicine related stomach disease?
    a. stomach ulcer
    b. heart burn
    c. gastroenteritis
    d. none

12. How many cigarettes do you smoke per day?
    a. below 5
    b. between 5 and 10
    c. more than 10
    d. never
    e. I quit. I used to smoke but I quit now.

13. How much do you drink per week?
    a. 2 glasses of wine
    b. one bottle of beer
    c. more than above
    d. never
    e. quit
14. What kinds of supplementation do you take?
   a. Vitamin
   b. mineral
   c. aloe vera
   d. royal jelly
   e. ginseng
   f. other __________
   g. none

15. In your opinion, you think you eat more Korean food or American food?
   a. Korean food
   b. American food
   c. same amount

16. How often do you substitute non-Korean ingredients in your Korean recipes?
   a. never
   b. some of the time
   c. most of the time
   d. all the time

17. Are you cooking traditional Korean foods the same way you did in Korea?
   a. yes
   b. no
   c. most of the time
   d. some time

18. If you answered no to the last question, what changes have you made in preparing and cooking traditional Korean foods?
   a. change spice (i.e. red pepper powder → black pepper powder)
   b. cabbage type (i.e. Chinese cabbage → Napa, cabbage)
   c. change meat (i.e. pork → beef)
   d. change red pepper paste → soy bean paste

19. Do you believe that your eating habit have changed since coming to the United States?
   a. yes
   b. no

20. Has your consumption of fast foods (e.g. McDonald’s, Wendy’s, etc) and/or convenient food items (e.g. frozen dinner, potato chips, ready-to-eat processed food items candy bars, etc.) increased since your immigration to the United States?
   a. yes
   b. no

146
Thank you for your cooperation.

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>serving size</th>
<th>2 day</th>
<th>serving size</th>
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</table>

147
Korean – survey

1. What is your gender?
   A: male
   b: female

2. What is your age in years? __________

3. Where do you live (please circle your answer)?
   a. large city (over 100,000)
   b. medium city (50,000-100,000)
   c. small city (10,000-49,000)
   d. town (2,500-9,999)

4. What’s your weight and height?
   a. wt : __________
   b. ht : __________

5. Please circle the highest grade in school you have completed:
   1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  +

6. What is /was your occupation when employed or before retirement?

7. Are you on a special diet?
   a. no
   b. yes
   *if yes, please, encircle the relevant item below:
   a. weight loss
   b. diabetic diet
   c. vegetarian
   d. low salt
   e. low cholesterol/fat
   f. weight gain
   g. other

8. What kinds of health problems do you have?
   a. none
   b. heart condition
   c. obesity
   d. high blood pressure
   e. diabetes
   f. cancer – liver, lung, stomach, other
   g. other
9. Do you have relatives with a stomach cancer?
   a. yes
   b. no

10. What do you think is the main cause of the cancer?
    a. Kimchi
    b. salted food
    c. smoked meat (i.e. burned meat)

11. Have you taken any medicine related stomach disease?
    a. stomach ulcer
    b. heart burn
    c. gastroenteritis
    d. nothing

12. How many cigarettes do you smoke per day?
    a. below 5
    b. between 5 and 10
    c. more than 10
    d. never
    e. I quit. I used to smoke but I quit now.

13. How much do you drink per week?
    a. 2 glass of wine
    b. one bottle of beer
    c. more than above
    d. never
    e. quit

14. What kinds of supplementation do you take?
    a. Vitamin
    b. Mineral
    c. aloevera
    d. royal jelly
    e. ginseng
    f. other __________
    g. none

15. Has your consumption of fast foods (e.g. McDonald’s, Wendy’s, etc) and/or convenient food items (e.g. frozen dinner, potato chips, ready-to-eat processed food items candy bars, etc.) increased since fast food restaurant open a lot?
    a. yes
    b. no
Thank you for your cooperation.

<table>
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<th>1 day</th>
<th>serving size</th>
<th>2 day</th>
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<td>breakfast</td>
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Appendix B

KOREAN SURVEY QUESTIONNAIRE
설문지
가장 합당하고 생각되시는 답에 동그라미를 해주시기 바랍니다.

1. 성별이 무엇입니까?
   1. 남성
   2. 여성

2. 연세는 어떻게 되는 지요?

3. 어떤 곳에 살고 계시는 지요?
   1) 대도시 (100,000)
   2) 도시 (50,000 - 100,000)
   3) 소도시 (10,000 - 49,000)
   4) 읍 (2,500 - 9,999)

4. 신장과 체중은 어떤지요?
   신장_____
   체중_____

5. 최종 학력을 표시해 주세요.
   0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 *

6. 지금의 직장이나 퇴직전의 직업은 무엇인지요?

7. 특별한 식사 요법을 하시는 지요?
   1) 예
   2) 아니오

* 만약에 예 이번 적당한 항목에 동그라미를 해주시기 바랍니다.
   1) 체중 감소
   2) 당뇨병
   3) 제식주의
   4) 저염주의
   5) 저 지방 식사
   6) 체중 증가
   7) 기타

152
153

1. などを
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여태까지 도와주셔서 감사합니다. 여기에 2일간 잡수셨던 모든 것을 상세히 기록해 주시기 바랍니다.

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설문지
가장 합당하다고 생각되시는 답에 동그라미를 체주시기 바랍니다.
1. 성별이 무엇인가?
   가. 남성
   나. 여성

2. 연세는 어떻게 되는 지조?

3. 어떤 곳에 살고 계실는 지조?
   가) 대도시(100,000)
   나) 도시 (50,000 - 100,000)
   다) 소도시 (10,000 - 49,000)
   라) 읍 (2,500 - 9,999)

4. 신장과 체중은 어떻게 지조?
   신장______
   체중______

5. 최종 학력을 표시해 주세요.
   0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 +

6. 지금의 직장이나 퇴직전의 직업은 무엇인가요?

7. 특별한 식사 요법을 하시는 지조?
   가) 예
   나) 아니오

8. 만약에 예 이런 적당한 항목에 동그라미를 체주시기 바랍니다.
   가) 체중 감소
   나) 당뇨병
   다) 체직주의
   라) 저염주의
   마) 저 지방 식사
   바) 체중 증가
   사) 기타

9. 무슨 종류의 병이 있는 지조?
   가) 무
   나) 심장병
   다) 비만
   라) 고혈압
   마) 당뇨병
   바) 암 - 간, 위장, 혈관, 기타
   사) 기타

10. 신체 중에 위장 암이 있으신 분이 계신 지조?
    가) 예
    나) 아니오
11. 위장 임의 주요 요인이 무엇이라고 생각하시는지요?
   가) 검지
   나) 소금에 젖인 옷식
   다) 탄 불고기

12. 위장병에 관련된 약을 드신 경우가 있으신지요?
   가) 위궤양
   나) 숙 쓰림
   다) 위하수
   라) 위장염
   마) 없음

13. 하루에 답례품 몇 개나 피우시는지요?
   가) 5 개미만
   나) 5~10개피 사이
   다) 10개피보다 많이
   라) 전혀 피우지 않음
   마) 지금은 몰랐으나 과거에 피웠음

14. 술은 일 주일에 얼마나 하시는지요?
   가) 와인 2 잔
   나) 맥주 한병
   다) 위보다 많이
   라) 전혀 안 함
   마) 몰랐음

15. 영양제를 잡수시는지요?
   가) 비타민 과 무기질
   나) 알로에 베라
   다) 류알제리
   라) 인삼
   마) 기타
   바) 전혀 먹지 않음

16. 혈비거나 면동 식품을 섭취하는 비율이 증가하였는지요?
   가) 예
   나) 아니오
여태까지 도와주셔서 감사합니다. 여기에 2일간 잡수셨던 모든 것을 상세히 기록해 주시기 바랍니다.

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158
Appendix C

PROTOCOL FOR ENZYME ASSAY
ASSAY FOR TOTAL GLUTATHIONE IN FOODS

Reagents: 0.1 M phosphate, 5mM EDTA buffer at pH 7.5

   Ellman's reagent -5,5' dithiobis-nitorbenzoic acid, DTNB-0.0148 g/25ml buffer

   Glutathione (GSH) -0.02 g/50ml 5% Perchloric acid diluted from 70%

   NADPH- 0.0042 g/2.5 ml buffer

   Glutathione reductase (type III from yeast) - 15 µl / 10ml buffer

   5 % trichloroacetic acid

1. Selected Korean foods were prepared according to most common cooking method in Korea. (steamed or boiled for 15 min)

2. Food samples were weighed 1g and homogenized (Kinematica Ch-6010, Kriens-Lu) in a 3ml of 5% perchloric acid for 20sec.

3. Homogenized sample were centrifuged (Beckman Avanti J25) at 10,000 rpm for 5 min and diluted 1/4.

4. The obtained supernatant was used as sample.

5. The reaction mixture consisted of 425µl DTNB, 50µl sample or standard, 425µl glutathione reductase, and 100µl NADPH.

6. The increase in absorbance (Beckman DU-40) at 412 nm was recorded over 5 min.

7. The concentration of GSH in the samples was calculated from the standard curve.
ASSAY FOR TOTAL GLUTATHIONE

Reagents: 0.1 M phosphate, 5mM EDTA buffer at pH 7.5

Ellman's reagent -5,5' dithiobis-nitorbenzoic acid, DTNB-0.0148 g/25ml buffer

Glutathione (GSH) -0.02 g/50ml 5% TCA, diluted 1/20, 1/160

NADPH- 0.0042 g/2.5 ml buffer

Glutathione reductase (type III from yeast) - 15 µl / 10ml buffer

5 % trichloroacetic acid

1. Rats were sacrificed by the carbon dioxide inhalation.

2. Removed Liver, stomach, small intestine and colon were frozen in liquid nitrogen and stored at -80°C until analysis.

3. Approximately 0.5 g of tissue, measured accurately, was homogenized in 3 ml of 5% TCA (kept in ice) with an homogenized with Kinematica GmbH (CH6010) and then centrifuged (Beckman Avanti J25) at 10,000 rpm for 5 min and diluted 1/4.

4. The supernatant fraction was decanted 80µl DTNB solution, 50µl GSH standard or sample, 80µl GSSG reductase and 30µl NADPH solution.

5. The DTNB solution, the GSH sample and the reductase solution were incubated at 25°C for 2 min.

6. The NADPH was added to begin the reaction.

7. The increase in absorbance (Spectra Max 250) at 412 nm was recorded over 5 min.

8. The concentration of GSH in the samples was calculated from the standard curve.
ASSAY FOR GSH PEROXIDASE

Reagents: 100mM Hepes, 0.1mM EDTA buffer at pH 7.5
0.01 M phosphate, 0.15 EDTA buffer at pH 7.4
Glutathione (GSH) 0.0154 g/ 25 ml 0.01 N HCl
NADPH - 0.0022 g/ 4.8 ml Hepes buffer
GSH reductase - Type III from yeast (15 μl /9.6 ml buffer)
\( t \)-butyl hydroperoxide – 16 μl /10 ml ethanol

1. Liver and other tissues (0.5g) were homogenized in 8ml ice-cold monobasic phosphate buffer pH 7.4.
2. The samples were centrifuged at 100,000 \( \times \) g for 1 hour.
3. The supernatant fraction was decanted and diluted and used as the source of peroxidase.
4. The preincubation mixture contained 10 μl GSH, 40 μl NADPH, 40 μl GSH reductase, 66 μl Hepes buffer and 10μl of sample and was incubated at 37 °C for 5 min.
5. 4 μl of \( t \)-butyl hydroperoxide was added to begin the reaction.
6. The decrease in absorbance as NADPH was oxidized was monitored at 340nm for 5 min.
7. The extinction coefficient of \( 6.3 \times 10^2 \) 1/mol mm for NADPH was used to calculate the activity of the peroxidase.
Figure 1. Change in absorbance versus protein concentration for glutathione peroxidase activity, using conditions as detailed previously in the methods. $r = .982$
ASSAY FOR GSSG REDUCTASE

Reagents: 0.1 M phosphate, 0.5 mM EDTA buffer at pH 7.6

0.01 M Phosphate, 0.15 M KCl buffer at pH 7.4

Glutathione disulfide (GSSG)-0.0153 g/10ml buffer pH 7.6

NADPH 0.0021 g/10 ml buffer pH 7.6

1. Liver and other tissue (0.5g) were homogenized in 8ml of phosphate buffer pH 7.4 and then centrifuged at 100,000 x g for 1h.

2. The supernatant fraction was decanted, diluted 1/5 and used as the source of the reductase.

3. The total GSSG solution and the total NADPH solution were mixed with 2.5ml of additional buffer.

4. 180 μl of this solution was used in each incubation.

5. The solution was preincubated for 3 min at 30° C then 20μl of the supernatant fraction were added to begin the reaction.

6. The decrease in absorbance of NADPH as it becomes oxidized was monitored at 340nm over 5 min.

7. The extinction coefficient of NADPH, $6.3 \times 10^2$ 1/mol mm, was used to calculate the activity of the reductase.
Figure 2. Change in absorbance versus protein concentration for glutathione reductase activity, using conditions as detailed previously in the methods. $r = 0.951$
ASSAY FOR GSH S-TRANSFERASE

Reagents: 0.01M phosphate, 0.15 M KCl buffer KCl buffer pH 7.4

0.1 M phosphate, 0.1 mM EDTA buffer pH 7.0

Glutathione (GSH) -0.0462 g/ 10ml 0.01 N HCl

1-chloro-2,4,-dinitrobenzene (CDNB)-0.038 g/5 ml DMSO

Dimethyl sulfoxide (DMSO)

33% trichloroacetic acid (TCA)

1. Liver and other tissue (0.5g) were homogenized in 8ml ice-cold phosphate buffer at pH 7.4.

2. The samples were centrifuged (Beckman L7-65) at 100,000 × g for 1 h at 4°C.

3. The supernatant fraction was decanted, diluted 1/25-1/50 and used as the source of the transferase.

4. The 400μl incubation mixture contained 320μl phosphate buffer pH 7.0, 25μl GSH, 25μl sample.

5. The buffer, GSH and sample were preincubated at 37° C for 2 min in a shaking water bath.

6. 5μl of CDNB was added with mixing to begin the reaction in the water bath.

7. The reaction was stopped 5 min later with the addition of 25μl 33% TCA.

8. The tubes were centrifuged in a table-top centrifuge (Hermle Labnet Z233M) at 600 rpm for 5 min.
9. The supernatant fraction was decanted and the absorbance (Spectra Max 250) measured at 340nm.

10. The complete reaction mixture without a protein sample was used as a control for the non-enzymatic reaction.

11. The GSH S-transferase activity was calculated using the extinction coefficient 9.6mM$^{-1}$ cm$^{-1}$ (Habig et al.1974).
Figure 3. Changes in absorbance versus protein concentration for glutathione s-transferase activity, using conditions as detailed previously in the methods. $r = 0.98$