EFFECTS OF THE PHYTOCHEMICALS QUERCETIN AND GENISTEIN ON THE

PHASE I AND PHASE II ENZYME ACTIVITIES

by

DAWN MARIE PENN

(Under the Direction of JOAN G. FISCHER)

ABSTRACT

Quercetin and genistein are flavonoids commonly found in fruits and vegetables that may protect against the development of chronic diseases, such as cancer. They protect against cellular damage, through one possible mechanism of inducing the anti-cancer Phase II detoxification enzymes without concurrently stimulating Phase I enzyme activity. The objective of this study was to determine whether quercetin and genistein increase activity of the Phase II enzymes, Glutathione-s-Transferase and Quinone Reductase, in a dose response manner while decreasing or having no effect on the Phase I enzyme, CytochromeP4501A1. Flavonoids were fed to rats, and enzyme activity was measured in various tissues at 14 and 28d. The results demonstrated the ability of both flavonoids to increase Phase II enzyme activity while either decreasing or having no effect on CytochromeP4501A1 activity.

INDEX WORDS: Flavonoids, Quercetin, Genistein, Glutathione-S-Transferase, Quinone Reductase, Cytochrome P450 1A1

EFFECTS OF THE PHYTOCHEMICALS QUERCETIN AND GENISTEIN ON THE

PHASE I AND PHASE II ENZYME ACTIVITIES

by

DAWN MARIE PENN

B.S., Penn State University, 2000

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003

EFFECTS OF THE PHYTOCHEMICALS QUERCETIN AND GENISTEIN ON THE

PHASE I AND PHASE II ENZYME ACTIVITIES

by

DAWN MARIE PENN

Major Professor: Joan G. Fischer

Committee: Julie Coffield
                     Ruth Harris

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2003

DEDICATION

This thesis is dedicated to the memory of Steve Power, who unexpectedly made it all so real. Steve lived his life trying to make it better for others through his humor and unselfish ways. I hope to always live my life by his example and appreciate the simplest of things, just as Steve always had.

ACKNOWLEDGEMENTS

There are a number of people that I would like to thank for their time, knowledge, support, and encouragement as I worked to complete this project.

Dr. Joan Fischer: I was very fortunate to have Dr. Fischer as my major professor. I have always appreciated her willingness to take a chance on me and give me the opportunity to work on this project. Without her constant patience, encouragement, and willingness to listen, I would not have had such an enjoyable experience as I did over the past few of years working in her lab.

Dr. Julie Coffield: Dr. Coffield was kind enough to help me out by unexpectedly joining my committee so late in the process. I have always had so much respect for Dr. Coffield as a scientist and as a person. There have been several times that I have turned to her for help and advice. Her kind heart, willingness to listen, and practical advice have always been appreciated.

Dr. Ruth Harris: Dr. Harris has been a valuable asset to my graduate career. Her knowledge has saved me a lot of time as I worked to complete my project. She has been most willing to help me out in various situations, and has been very patient in answering my endless questions about lab or class work. I am truly grateful for her encouragement, the opportunities, and the advice that she has provided me during my time here.

Joyce Power: I am so thankful to Joyce for everything that she has done for me concerning her roles as a lab tech and especially as one of my best friends. Joyce often went beyond what was required of her job by spending some nights and weekends in the

lab with me. Without her help it may not have been possible to complete my project in the time that it was. My experience in Georgia would not have been as enjoyable and fun if Joyce were not a part of it. I have tormented Joyce by making her the focus of many practical jokes and have tortured her with hour's worth of my stories. In return she has become one of my most loyal friends and is even willing to provide me with fantastic home cooked meals.

Dr. Michael Mouat: In the earlier stages of my project there was hardly a day that went by that I didn't ask Michael for his help. He sacrificed much of his time to answer my questions and help me with some of the hands on work of my project. His kind and patient ways make him so approachable and easy to work with. I am very grateful to Michael for his time, knowledge, and advice that he has given me throughout my graduate career.

Dr. Srinivasa Muralidhara (SM): Without the help of SM about one third of my project would not have been completed. SM was kind enough to give me full access to equipment, and chemicals needed to complete one of the enzyme assays. He also sacrificed much of his time showing me how to run the assay and answer my many questions.

My Parents and My Brothers: I would like to thank my parents, Ron and Fran Penn, for giving me the opportunity to continue my education and for giving me the freedom to choose the directions I wish to live my life. Without their encouragement and support throughout my lifetime I never would have made it this far or have been so happy living my life as I've pursued my opportunities. I would also like to thank my brothers, Chad and Todd. They have always encouraged me to get out of my comfort zone and take a

chance. Without their practical advice I may never have made my way to graduate

school.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2

Page

CHAPTER 1

INTRODUCTION

For decades it has been demonstrated that the consumption of various plant

substances is protective against the development and progression of chronic diseases

(Steinmetz and Potter, 1996; Van Duyn and Pivonka, 2000). This protection is accredited

to the nutritive and non-nutritive components of the plant. Both i*n vitro* and *in vivo*

animal studies have demonstrated that non-nutritive components, known as

phytochemicals, are capable of inhibiting various stages of the cancer process

(Wattenberg, 1992). Phytochemicals can be found in a variety of plant sources such as

vegetables, fruits, nuts, seeds, cereals, and legumes (Kuhnau, 1976). A class of

phytochemicals called flavonoids is further divided into flavonols and isoflavones. The

flavonol, quercetin, is commonly found in fruits and vegetables, and the isoflavone,

genistein, is abundant in soybeans.   Both flavonoids are bioactive compounds capable of

protecting against cellular damage. Various mechanisms have been proposed for their

protection against carcinogenesis (Setchell, 1998; Singhal, 1995; Scambia, 1994;

Yoshida, 1992; Agullo, 1994). One proposed mechanism is the modulation of

detoxification enzymes. Phase I (PI) and PII (PI) enzymes are enzyme systems within

organisms that are responsible for converting fat-soluble xenobiotics into water-soluble

compounds, which is essential for their excretion (Klaassen et al., 1986). Detoxification

by PII enzymes occurs through the conjugation of a non-reactive water-soluble

component to a toxic lipophilic compound, resulting in its harmless removal from the

organism. PI enzymes are typically responsible for bioactivation, which can result in the

formation of reactive electrophilic compounds that can bind to macromolecules and lead to mutagenesis (Klaassen et al., 1986). The PII enzymes prevent binding of the reactive PI products by conjugating them before they inflict damage. Therefore a higher level of PII enzymes is theoretically protective against cancer, and can be accomplished by inducing PII activity only, which is known as mono-functional enzyme induction (Talalay, 2000) or decreasing PI enzyme activity. Quercetin and genistein have both demonstrated the ability to increase PII enzymes, which is believed to be protective against cancer development (Appelt and Reicks, 1997; Appelt and Reicks, 1999; Breinholt et al., 1999; Fischer and Fisher, 2000; Mikulcik and Fischer, 2001; Mirsalis et al., 1993; Wei et al., 1995)

I tested the hypothesis that the dietary administration of the aglycone flavonoids, genistein and quercetin, would mono-functionally increase and maintain the activity of the PII detoxification enzymes glutathione -S- transferase (GST), and quinone reductase (QR) over a period of time in the liver, kidney, colon, and lungs of male Sprague-Dawley rats. The objectives of this study were: 1) to investigate if genistein and quercetin could mono-functionally induce the activity of the PII enzymes, GST and QR, without inducing the activity of the PI enzyme CYP1A1. 2) To determine if enzyme activity increases in a dose response manner. 3) To examine if change in enzyme activity is maintained over a 14 to 28 d time period. Investigating whether an aglycone flavonoid is capable of producing a positive biological response is advantageous over using a whole food because it will enhance the understanding of how one component of the whole food contributes to the mechanism of protection, or whether a flavonoid is capable of

producing a positive biological response without the intricate chemical mixture of a whole food.

The effect of phytochemicals on biotransformation enzymes is a relatively new area of research and there is little *in vivo* data on the appropriate doses and duration of treatment needed to increase enzyme activity. Therefore an important question to answer, to understand the actions of quercetin and genistein, is what concentration of the flavonoids is needed to elicit a response. This study suggests that both quercetin and genistein are mono-functional PII enzyme inducers. Their effects on enzyme activity were quite variable and depended on tissue, dose, compound and length of consumption. The primary finding was a dose response increase in QR activity, with a decreased CYP1A1 activity, in the colon of quercetin treated animals. Key findings were 1) there was a dose response increase in mucosa QR activity after 14 and 28d of quercetin supplemented animals, 2) we observed a decrease in 14d liver and kidney, and 28d lung QR activity at all three doses of genistein, and 3) only the highest dose of quercetin and the lower doses of genistein were capable of increasing liver GST activity after 14d.

The administered low and medium doses of genistein were similar in concentration to amounts typically consumed in an Asian diet per kilogram body weight (Wang and Murphy, 1996). Consumption of genistein, in a concentration able to mono-functionally induce enzyme activity, could contribute to its protective action against cancer, which supports the notion that a diet rich in fruits and vegetables has beneficial effects. The doses of quercetin used were far above achievable human consumption even with supplements. However, the lowest dose of quercetin did demonstrate that it was capable of inducing colon QR and decreasing CYP1A1 activity by a 2-fold

difference from control, which could be important in the pathology of colon cancer. It is speculative, but it could be possible that the consumption of a combination of flavonoids may exert a synergistic effect on the inducing capacity of quercetin, and an increase in QR activity could be possible with the typical intake of flavonoids in the human diet.

This is the first *in vivo* study to test aglycone quercetin's effect on colon QR and CYP1A1 activity without the addition of a carcinogen. Maintaining a high physiological concentration of flavonoids, capable of mono-functional enzyme induction, could be key in protecting against cancer, in that the body will be prepared to quickly eliminate carcinogens resulting in less cellular damage.

CHAPTER 2

LITERATURE REVIEW

<u>Cancer</u>

Hippocrates was the first to describe cancer as a crab like structure due to the physical presentation of long distended veins extending into the surrounding tissue (Pitot, 1986). Cancer is a negative consequence of DNA being able to evolve and mutate, providing humans with the ability to adapt to and to survive in various environments (WCR and AICR, 1997). Cancer is caused, in almost all situations, by mutation or by some other abnormal activation of cellular genes that control cell growth and cell mitosis. Only a minute fraction of the cells that are mutated in the body ever lead to cancer. The reason for the low rate of occurrence is that several systems within the body are capable of either destroying or repairing any mutated cells. Preventing mutations could decrease the formation of abnormal proteins responsible for signaling inappropriate cell proliferation or inhibition (Guyton and Hall, 2000). The most prevalent differences between a cancer cell and a normal cell are: the cancer cells do not respond to the internal signals that inhibit cell growth, cancer cells are less adhesive than normal cells and can wander freely through circulation, and cancer cells release angiogenic factors that promote the development of a vascular network to provide a constant supply of nutrients to the growing tumor (Guyton and Hall, 2000). Cancer eventually kills because the growing mass interrupts the usual functions of the organs and/or because the increased number of cancer cells utilize the nutrients from the blood supply leaving

normal cells to die (Guyton and Hall, 2000). Most organisms that live long enough will eventually develop cancer. Therefore it is the premature development of cancer that is of concern in the United States. Currently, cancer is the second leading cause of death in the United States, and is responsible for 1 in every 4 deaths (USDHHS et al., 2001). Overall, it is estimated that about 1,334,100 new cancer cases will be diagnosed and 556,500 cancer deaths will occur in the US in 2003 (ACS, 2002). Environmental risk factors are responsible for 50-75% of all cancer deaths in the United States (Reis et al., 1999) compared to 5% of deaths associated with only genetic factors (Rock et al., 2000). Environmental risks include smoking, diet, infectious diseases, and exposure to chemicals and radiation (ACS, 2002). The molecular pathology of cancer is a multi-step process involving the accumulation of genetic changes that result from the interaction between genetics and environmental factors, such as diet (Lai et al., 1999; Perera, 1997). Certain factors in the diet have been found to increase mutations and therefore research continues to determine the link between nutritional factors and cancer risk.

Biotransformation Enzymes

Over time, organisms have been exposed to an increased number of foreign chemicals, or xenobiotics. An elaborate system of enzymes has developed in many tissues to metabolize endogenous and foreign lipophilic compounds. The PI enzymes are typically found within the endoplasmic reticulum of cells (Williams et al., 2000). Their purpose is to either expose or bind a functional group to a compound through the actions of hydrolysis, reduction, or oxidation. Their action results in a reactive hydrophilic compound (Oesch and Arand, 1999), that can induce PII enzymes. The PII enzymes

catalyze the binding of a water-soluble compound to the electrophile, resulting in a harmless hydrophilic product. The PII enzymes, or conjugating enzymes, are typically located within the cytosol of cells (Oesch and Arand, 1999).

The ability of a chemical to induce both the PI and PII enzymes is called bi-functional induction. This type of induction promotes a balance between the two classes of enzymes and results in chemical excretion. Excreting a chemical is protective to an organism because the time a toxin remains in the circulation is shorter, but increasing only the PII enzyme activity would be an ideal protection method because PII enzyme conjugation usually results in the formation of an inactive compound. A chemical that is able to induce the activity of the PII enzymes without increasing PI activity is called a mono-functional inducer.

In general, xenobiotics are lipophilic compounds that have no nutritive value, do not produce energy or build tissues, and may be a reactive compound that can cause cellular damage (Klaassen et al., 1986). These lipophilic substances occur in the environment, and are also endogenously produced (Klaassen et al., 1986). Lipophilic xenobiotics from the environment are internalized through ingestion, inhalation, and dermal exposure. Examples of endogenous lipophilic compounds that need to be converted to water-soluble product, in order to be excreted include hormones, neurotransmitters and quinones formed from byproducts of the electron transport chain (Talalay, 2000). Once the fat-soluble compound reaches circulation they can easily diffuse through cell membranes and settle into tissues, particularly fat. A build up of xenobiotics can create a burden to the body and need to be excreted. Routes of excretion are through urine, bile, and for volatile chemicals, exhalation. To be excreted the

compound first needs to be converted into a water-soluble substance. The transformation

of the lipophilic compound to an excretable constituent is accomplished by either

detoxifying or bioactivating the xenobiotic. Detoxification results in the harmless

removal of a compound from the organism, whereas bioactivation results in the formation

of a reactive electrophilic compound that aggressively tries to fulfill its need for an

electron by binding to nucleophilic macromolecules, like DNA, RNA or phospholipids.

Binding to these macromolecules can lead to mutagenesis or lipid oxidation (Klaassen et

al., 1986). Determinination of whether a molecule will be activated or detoxified is

generally dependent on which enzyme system, the phase I or phase II, is activated (Oesch

and Arand, 1999). The overall effect of introducing a xenobiotic into an organism results

in induction of activity in one or both of the enzyme systems. The onset, magnitude, and

duration of enzyme activity varies with the inducing agent, its dose, species, strain, sex of

the animal, duration of exposure, and the tissue in which enzyme activity is being

measured (Williams et al., 2000). The inhibition of enzyme activity may occur when an

exogenous or endogenous compound decreases the ability of an enzyme system to

metabolize the chemical. Examples of inhibitory factors include agents that decrease

protein synthesis, decrease enzyme co-factors within tissues, and compete for the same

binding site of an enzyme (Williams et al., 2000). These many variables make predicting

a xenobiotic's effect on enzyme induction difficult.

The Phase II Xenobiotic Enzymes

        The conjugating activity of the PII enzymes requires certain conditions in order

to take place. First the xenobiotic must have an appropriate functional group exposed for

8

conjugation. Second, a substrate, such as glutathione, must be present for conjugation to the xenobiotic. If the xenobiotic does not have a functional group that will induce the PII enzymes then oxidation by PI enzymes may take place in order to expose or attach an appropriate functional group. Once these steps occur, the oxidized product and the co-substrate must be simultaneously available for conjugation in order to avoid the tissues being exposed to an electrophile (Hayes et al., 1996).   The subsequent action of the PII enzymes following the activity of the PI enzymes is essential is generating a compound water-soluble enough for excretion. The products of the PI enzymes are more water-soluble than their parent molecules. Some metabolites are however still quite fat-soluble and the conjugation to water-soluble proteins further increases their hydrophilicity to ensure excretion in the urine or bile. PII enzymes include several enzymes or families of enzymes: the sulfotransferases, UDP-glucuronosyltransferase, epoxide hydrolase, glutathione -S- transferases (GST), quinone reductases (QR) and amine oxidases are examples. Key PII enzymes involved in the detoxification process include GST and QR (Klaassen et al., 1986; Oesch and Arand, 1999). The ubiquitous presence of these enzymes allows for xenobiotic metabolism to occur in all organs and tissues. Quantitatively, the liver is the most important organ involved in detoxification, and it contains high concentrations of enzymes because of its involvement in "first pass effect" of inactivating the xenobiotic before it reaches the systemic circulation (Oesch and Arand, 1999). Comparatively, the metabolizing capacity of the extrahepatic tissues is inferior to the liver.   They are, however, important to extrahepatic organs because the enzymes have a primary role in limiting the toxicity of a compound within those organs (Klaassen et al., 1986).

<u>Glutathione-S-Transferase</u>

GSTs are located in the microsomal and cytosolic fractions of the cell, but are more active in the cytosol. There are five GST gene families (Strange et al., 2000). They are present throughout the body with greatest activity in the liver, kidney, intestine, testis, and adrenal glands (Klaassen et al., 1986). GSTs are induced by compounds metabolized by PI enzymes and phenolic compounds (Oesch and Arand, 1999). The enzyme's highly specific active site for binding glutathione is called the G-site, while the less selective binding site for hydrophobic xenobiotics is called the H-site. The broad capacity of the enzyme's xenobiotic binding site renders GSTs significant protectors against chemical carcinogenesis (Hayes et al., 1996). The binding cofactor that is utilized by this enzyme is the tripeptide glutathione, which is composed of glycine, glutamic acid, and cysteine (Klaassen et al., 1986). The glutathione transferases catalyze the transfer of the nucleophilic glutathione thiolate on to the electron deficient atom of the somewhat hydrophobic, electrophilic xenobiotic. Conjugation results in the formation of a water-soluble metabolite. This compound is actively transported from the cell, usually by the type of transport proteins known as multi-drug resistance proteins, and is eventually transferred to the kidney. In the kidney the glutamate and glycine are cleaved from the complex. The remaining cysteinyl conjugate is processed further to form the final metabolite mercapturic acid, which is excreted in urine. Since glutathione is a large molecule, the conjugated xenobiotic may be secreted into the bile and excreted in the feces, but it is frequently excreted in the urine (Hayes et al., 1996).

Quinone Reductase

This enzyme is formally named NAD(P)H: quinone acceptor oxidoreductase. QR is a cytosolic enzyme that is present in the lung, breast, colon, liver, kidney, vascular endothelium, adipocytes, corneal and lens epithelium, and optic nerves and nerve fibers (Ross, 2000; Talalay, 2000). Once induced, QR carries out a 2-electron reduction of reactive quinones. Quinones are reduced to electrophilic semi-quinones, which are reduced to the stable hydroquinone. If the semi-quinone is not reduced it undergoes redox cycling and generates reactive oxygen species (ROS) (Cadenas, 1995; Talalay, 2000). The transfer of the second electron produces hydroquinone, which can be conjugated with glucuronic acid or glutathione by the action of the PII enzymes uridine diphosphate glucuronosyl transferase or GST (Talalay et al., 1988). The reducing activities of QR also spare the cellular supply of sulfhydryl groups because the reactive quinones are not present to deplete these. This is important because sulfur is a compound needed to form glutathione (Talalay, 2000). Inducers of QR include antioxidants, planar aromatics, oxidants, heavy metals, UV light, ionizing radiation, and the metabolites of phenolic antioxidants (Jaiswal, 2000). These secondary metabolites of polyphenols can induce the mono-functional induction of QR and are believed to all share a common structure that causes the induction (Talalay et al., 1988). The common intermediate must have the capability of being oxidized to a quinone in order for QR to carry out its 2-electron reduction. It has been found that the orientation of phenols in the para (1,4) or ortho (1,2) positions can be oxidized to quinones. This finding was consistent with the requirement that QR inductions were dependent on the ability of the inducers to undergo one or two electron oxidation/reduction reactions therefore inducing QR (Talalay, 2000).

Why these structures signal PII induction is unclear, but these structures are essential for mono-functional enzyme induction (Talalay, 2000).

The Phase I Xenobiotic Enzymes

The phase I transformation or functionalization step in the metabolism of xenobiotics is carried out by several categories of phase I enzymes. These are the cytochrome P450 dependent monooxygenases (CYP), the flavin-containing monoxygenases, the monoamine oxidases, and the cyclooxygenases. The transformations that they carry out, in order of most importance, are oxidation, hydrolysis, and reduction (Williams, 2000). All of these processes introduce oxygen into, or remove electrons from, the available substrates (Guengerich, 1993). The quantitatively most important phase I enzyme family is the CYP superfamily. Their evolution into a superfamily enabled the CYP450 system to evolve into 14 different families that have the capacity to metabolize a vast amount of exogenous and endogenus compounds (Anzenbacher and Azenbacherova, 2001). It is believed that these enzymes are needed to metabolize and detoxify chemicals found in plants. The plant-animal "warfare" hypothesis has been used to explain the evolution of CYPs. This hypothesis surmises that plant produced toxins killed their predators and eventually these animals evolved the CYPs to detoxify toxins (Guengerich, 1993). The CYP superfamily continues to grow as humans are exposed to new chemicals and pollutants. Although humans are able to generate new isozymes to facilitate the excretion of xenobiotics to benefit the organism, chemical carcinogenesis still exists. Chemical carcinogenesis occurs because the CYP enzymes typically activate chemicals introduced into the

organism and because new chemicals are being produced faster than the metabolizing

enzymes evolve (Guengerich, 1993). The general reaction of the CYP mediated oxidation

of xenobiotics occurs in the following steps. The xenobiotic binds to the cytochrome

hemoprotein at a substrate binding site on the protein and alters its shape to allow for the

transfer of electrons from NADPH to the heme molecule. The reduction of heme iron,

from ferric to ferrous iron allows molecular oxygen (O_2) to bind to the iron within the

cytochrome xenobiotic complex. The donation of another electron splits the molecular

oxygen and one oxygen atom is inserted into the xenobiotic and the second molecule of

oxygen is reduced and forms water. The remaining product is an oxidized xenobiotic.

The identity of the molecule that is oxidized within the xenobiotic determines the nature

of the reaction, e.g. epoxidation, N, S, C oxidation, aromatic hydroxylation. The CYP

system can metabolize a wide diversity of substrates that range from 42 to 1203 in

molecular weight (Ioannides, 1999).

Cytochrome P450 1A1

CYP1A1 is the first enzyme in the subfamily A of the CYP family 1 (Whitlock,

1999). CYP1A1 is a CYP isozyme that activates the procarcinogens to which humans

are often exposed. Polycyclic aromatic hydrocarbons, benzene, and theophylline are

chemicals commonly ingested or inhaled and are metabolized to ultimate carcinogens by

CYP1A1 (Oesch and Arand, 1999). They are introduced into the body as chemically

inert compounds, but once CYP1A1 metabolism takes place they are transformed into

mutagenic metabolites (Hayes et al., 1996). This mutagenic conversion of the pollutants

is why CYP1A1 activity is thought to have a role in the development of lung cancer

(Anzenbacher and Azenbacherova, 2001). CYP1A1 can be found in many organs

throughout the body including the lungs, placenta, liver, heart, kidney, and brain

(Klaassen et al., 1986). Its activity in tissues is not expressed to any appreciable degree

in the absence of dietary or environmental inducers (Williams et al., 2000).

The Balance Between Phase I and Phase II Enzymes

The ability of the PII enzymes to inactivate the electrophilic products of PI

metabolism demonstrates the importance of the balance between the enzyme systems.

The risk of DNA mutation increases when there is an increase of PI enzymes over PII

enzymes (Oesch and Arand, 1999). The level of enzyme activity is influenced by a

variety of factors such as: metabolic state, chemical dose, time of day, gender, species,

and length of exposure (Oesch and Arand, 1999). Diet and metabolic state are key

factors because the action of the PII enzymes depends on substrate availability. In order

to form the protein that conjugates and deactivates the electrophile the availability of

nutrients is essential.

Mono or Bi-functioal Enzyme Induction

Determining whether a compound will mono or bi-functionally induce enzyme

activity is dependent upon what region of the gene is activated. Mono-functional

induction occurs without involving the aryl hydrocarbon receptor (Ah). When the

xenobiotic does not associate with the nuclear Ah receptor it instead directly binds to a

region of the gene different from the location of where the Ah complex would bind

(Guengerich, 1993; Jaiswal, 2000). When the xenobiotic does not bind to the Ah

receptor it instead directly binds to the region of the gene called the antioxidant response element (ARE). Inducing the ARE will increase the activity of certain PII enzymes only without increasing the activity of the PI enzymes. Transcriptional bi-functional induction of the mRNA coding for CYP1A1/A2 and certain PII enzymes can be initiated through a xenobiotic binding to the Ah. Bi-functional induction begins when a xenobiotic binds to the Ah/heat-shock protein 90 in the cytosol of a cell. The Ah-xenobiotic complex disassociates from the heat-shock protein and binds to another protein called the aryl hydrocarbon receptor nuclear transferase (ARNT) molecule. This complex is then transported into the nucleus of the cell where it binds to a specific region of the gene, called the xenobiotic response element (XRE). The binding permits other proteins to access the promoter region of the gene and synthesize mRNA for the PI enzymes CYP1A1/1A2 and the PII enzymes QR, GST, UDPGT, and aldehyde dehydrogenase. (Guengerich, 1993; Nebert et al., 2000; Schrenk, 1998) (**Figure 1**). XRE is induced by planar aromatics, polyhalogenated dibenzodioxins, dibenzofurans, and biphenyls (Shertzer et al., 1999). To induce the PII enzymes only, another region called the antioxidant response element (ARE) on the gene is activated. The binding of various proteins to the ARE regulate the expression of the PII enzymes. Induction of the PII enzymes through the ARE can be activated by planar aromatics and phenolic antioxidants, such as the flavonoids, and electrophiles and H_2O_2 (Dinkova-Lostova and Talalay, 2000). The proposed mechanism begins with the xenobiotic signaling the proteins Nrf2 and Keap1 to dissociate from one another. Nrf2 translocates into the nucleus and forms a heterodimer with c-Jun, which binds to the ARE resulting in the

induction of the ARE regulated gene which activates the transcription of certain PII enzymes, but not CYP enzymes (Jaiswal, 2000).

Diet and Cancer Development

Once a foreign lipophilic compound is absorbed it is converted into a water-soluble compound through the actions of the metabolizing enzymes described above. Mutations occur when the absorbed xenobiotic is a carcinogen, or a procarcinogen that is absorbed and converted to an ultimate carcinogen by PI enzyme activity. The reactive carcinogen begins the first stage of carcinogenesis, called initiation, which produces alterations in the genetic make-up of the cell through somatic alterations of oncogenes, tumor suppressor genes, and DNA-repair genes. Without successful repair or cell death the mutated cell enters the stage of promotion. Promotion is characterized by transforming the initiated cells into a population of cancer cells through proliferation of the altered cells. This stage is reversible by the same mechanisms as the initiation stage. Failure to cease the proliferation of the mutated cells allows the cells to enter the third stage, called progression. During this last stage the cancerous cells of the tumor are able to invade surrounding tissues and distant sites throughout the body. Thus, metastasis is associated with widespread DNA damage. The loss of cell function will eventually lead to a loss in organ function, which could result in death. Increasing the level of PII enzymes over PI is critical in preventing cancer by decreasing the risk of a cell being initiated and the advancement of a cell into promotion and progression. Introduction of a carcinogen into an organism with an abundant amount of PII enzymes will allow rapid and harmless excretion of the molecule without initiation. Cells that have reached either initiation or promotion can differentiate into normal cells through the conjugating

capacity of the PII enzymes and efficient action of repair mechanisms. By decreasing the number of cells being mutated the repair mechanisms will be able to repair the cellular damage before the altered cell has an opportunity to proliferate and metastasize.

Diet and metabolic state are key components in determining whether carcinogens are able to initiate cellular damage. The role of good nutrition is essential in preventing cancer. Through a healthy diet, rich in fruits and vegetables, a human can consume the macronutrients, micronutrients, and phytochemicals needed to synthesize and induce the protective enzymes and their substrates needed to prevent cellular injury. A diet rich in fruit and vegetables should result in an increased level of PII enzymes, which potentially may decrease the risk of cellular damage.

Fruit and Vegetable Intake and Cancer Risk

One of the earliest discoveries of the link between diet and cancer was by Peyton Rous, who in 1914 observed food consumption restriction delayed the development of tumor metastases in mice (WCR and AICR, 1997). Since that time, concrete evidence from epidemiological, clinical, and laboratory studies suggests that various dietary constituents have the capacity to influence the development of cancer, prognosis after diagnosis, and quality of life during cancer treatment (Rock et al., 2000). High consumption of vegetables and fruits has been found to be protective against the development of cancer.   A decrease in cancer risk for the lungs, mouth, pharynx, esophagus, stomach, colon, rectum, breast, pancreas, larynx, and bladder has been associated with high fruit and vegetable intake (Reis et al., 1999). The leading sites of cancer death projected for 2003, in the US, are lung and bronchus, prostate, breast, and colorectal cancers (ACS, 2002). Thus, benefits of consuming a diet rich in vegetables and

fruits has been recognized by many government agencies as they work to encourage people to expand their diet to include a variety of vegetables and fruit. Recommendations and goals established by the American Cancer Society (ACS, 2002), USDA (USDHHS, 2000), (Garza et al., 2000), Surgeon General (USDHH, 2000), and other organizations include the increased intake of plant sources to reduce cancer risk (Garza et al., 2000). In 2000 however, the American Cancer Society found that only 1 in 4 adults in the US reported eating 5 or more servings of vegetables and fruit per day (ACS, 2002). This statistic demonstrates that the population is far from meeting the recommendation of the American Institute for Cancer Research (AICR) to eat a variety of 5-10 servings of vegetables and fruits daily, which may prevent at least 20% of all cancer incidence (WCR and AICR, 1997). In contrast to the protective effects of plant foods, some components of the diet have been found to augment the incidence of certain types of cancer. These constituents include high amounts of dietary fat (IARC, 1988), red meat (Van Duyn and Pivonka, 2000), calories (Steinmetz and Potter, 1996), and alcohol (Bravo, 1998).

Vegetable and fruit intake is encouraged due to potential chemoprotective mechanisms of the vitamin, mineral, and non-nutritive matter in these foods. *In vitro* and *in vivo* studies have demonstrated that naturally occuring plant compounds are capable of inhibiting various stages of the cancer process (Wattenberg, 1992). Their protection against cancer and other chronic diseases involves interfering with the expression and/or activity of harmful substances by either initially preventing damage or repairing cellular injury. Many of these dietary agents have the capacity to engage in multiple chemoprotective actions. Vitamins C and E act as antioxidants by inhibiting the

destructive action of free radicals (Havsteen, 1983). Calcium and Vitamin D have the ability to reduce cell proliferation (Havsteen, 1983), and folic acid has a role in correcting DNA methylation imbalances (Kuhnau, 1976; Mitchell, 1998). Vitamin E and selenium have a role in restoring immune response (Havsteen, 1983), and selenium is also a co-factor for the antioxidant enzyme glutathione peroxidase (Havsteen, 1983; Mitchell, 1998). Calcium and fiber may bind, and thus decrease the absorption of carcinogens. Fiber also has a protective role by increasing transit time, which will reduce the amount of time that carcinogens are in contact with the colon (Hertog et al., 1993). The evidence that a diet rich in fruits and vegetables has the potential to reduce the risk of many cancers is strong, but it is not known which constituents of these foods are responsible for reduced risk (Willett, 2000).

Phytochemicals

The non-nutritive bioactive components of plants that contribute no calories and bestow positive benefits to health are termed phytochemicals. They are also known as phytonutrients and vitalimins (Herrmann, 1976). Examples of classes of phytochemicals include carotenoids, inositol phosphates, protease inhibitors, and polyphenols (Herrmann, 1976). They are essential to plants because of their role in plant morphology, growth, reproduction, and for protection against predators, oxidative stress and pathogens. Their concentrations vary between cultivars of the same species, environmental conditions, germination, degree of ripeness, and variety (Agullo et al., 1994). Phytochemicals can be found in a variety of plant sources such as vegetables, fruits, nuts, seeds, cereals, and legumes (Kuhnau, 1976), and in beverages like wine, beer, cider, tea, and cocoa

(Havsteen, 1983). Polyphenols are a class of phytochemicals, which are ubiquitous

within the plant kingdom, and are products derived from the secondary metabolism of

plants (Bravo, 1998). A portion of the sensory and nutritional qualities of plant foods and

beverages, such as astringency and bitterness, is due to the presence of polyphenols

(Bravo, 1998). Polyphenols have long been used industrially for the production of paints,

paper, cosmetics, and food additives. Typical medicinal uses of polyphenols are as

antibiotics, anti-diarrheal and anti-ulcer agents, and anti-inflammatory compounds

(Bravo, 1998).   The polyphenols can be divided into 10 different classes depending on

their basic chemical structure (Bravo, 1998). The bulk of polyphenols fall into the widely

distributed flavonoid class, which contains over 5,000 compounds that are further divided

into 13 different categories (Bravo, 1998). The different categories all share a similar

skeleton structure that is responsible for their numerous therapeutic effects (Herrmann,

1976). The common diphenylpropane structure is characterized by a benzene ring (A)

and a condensed six-member ring (C), which carries the phenyl ring (B), as a substituent

(**Figure 2**). The capacity of the basic flavonoid structure to conjugate hydroxyl groups,

methyl ethers, acetyl esters, and various sugars at different positions allows for the

formation of over 20,000,000 different flavonoids, which are classified into one of the 13

flavonoid subdivisions (Bravo, 1998).

The formation of flavonol glycosides depends normally on the action of light.

The glycosidic form of a phytochemical is the form in which it has a sugar molecule

attached to its phenolic ring. In general, the highest concentrations of polyphenols are

found in the leaves of a plant while trace amounts are present in the parts of the plant

below the soil (Herrmann, 1976). The location of the polyphenols within the leaves is

probably due to their role in photosynthesis. When photosynthesizing cells die, the flavonoids are released from the thylakoid membrane of the plant cell and appear in the plant juice (Havsteen, 1983).

The Flavonoids: Quercetin and Genistein

The estimated average consumption of the common flavonoids, quercetin, kaempferol, myricetin, apigenin, and luteolin is 23 mg/d (Hertog et al., 1993). Two important flavonoid classes abundantly found in the food supply are flavonols and isoflavones. It is the position of the benzenoid substituent (B ring) that divides the flavonoid class into the flavonols (2-positon) and isoflavonoids (3-postion) (**Figures 3 and 4**) (Havsteen, 1983). A flavonol that is commonly found in fruits and vegetables is quercetin, whereas the isoflavone genistein is abundant in legumes, particularly soy beans.

The aglycone form of quercetin (3,3',4',5,7-pentahydroxyflavone) differs from the general flavonoid structure by the presence of a hydroxyl group on B3', and a hydrogen on B5' (**Figure 3**). The preferred binding site of a sugar moiety is the 3-position, and much less frequently the 7-position (Herrmann, 1988). Sources of quercetin include: berries, olives, broccoli, brussel sprouts, lettuce, fruits, cranberry, tea, and red wine. Especially rich sources are onions and apples (Bravo, 1998; Herrmann, 1976). It has been estimated that quercetin intake of the general population is around 16 mg/day (Hertog et al., 1993). This flavonol has been found to inhibit proliferation of human cells from breast (Singhal, 1995), ovarian (Scambia, 1994), leukemic (Yoshida et al., 1992), and colon (Agullo et al., 1994) cancers. Both *in vivo* and *in vitro* studies has shown

21

quercetin capable of acting as an antioxidant and potentially reducing the risk of the development of chronic diseases by protecting low density lipoprotein (LDL) from oxidation (Breinholt et al., 1999; Catapano, 1997; Rice-Evans et al., 1996). Quercetin has demonstrated anti-inflammatory activities by inhibiting the release of prostaglandins from damaged tissues. The body releases prostaglandins, among other chemicals, in response to injury, which causes pain and inflammation. Quercetin can also protect against diabetic retinopathy, neuropathy, and nephropathy by inhibiting the enzyme aldose reductase. The action of this enzyme accumulates sorbitol, which has been linked to nerve, eye, and kidney damage in diabetes (Havsteen, 1983). It also influences the activity of the phase I and phase II enzymes to protect against cancer (Fischer and Fisher, 2000; Mikulcik and Fischer, 2001; Yannai et al., 1998).

Another flavonoid known to contribute many health benefits is the isoflavone genistein, which is found in soy. Isoflavonoids are found in plants of the subfamily *Papilionoideae* of the *Leguminosae*, which includes soybeans (Harborne, 1994). Soy has been studied extensively in the past decade because of the health benefits associated with the phytoestrogenic properties of its two major isoflavonoids, genistein and daidzein (Setchell, 1998). Soy has shown anti-cancer affects for breast, prostate, endometruim and colon cancers (Setchell, 1998). Anti-carcinogenic mechanisms of genistein include decreasing cell proliferation and transformation by inhibiting tyrosine kinase, or by altering the cell cycle via other mechanisms (Boersma, 2001). Tyrosine kinases are known to induce cell proliferation by phosphorylating tyrosine residues on proteins, such as growth factors involved in tumor cell signaling transduction and proliferation (Kurzer et al., 1997). Genistein may also prevent cellular damage through increasing the activity

of the phase II xenobiotic metabolizing enzymes in various tissues (Appelt and Reicks, 1997; Appelt and Reicks, 1999; Breinholt et al., 1999; Mirsalis et al., 1993; Wei et al., 1995). Various attributes of genistein may allow the compound to have a protective role against heart disease, diabetes, renal disease, cancer, osteoporosis, and symptoms of menopause. The isoflavone may reduce the risk of heart disease by functioning as an antioxidant, or increasing the activity of antioxidant enzymes to protect against LDL oxidation (Mitchell, 1998). The antioxidant properties and low glycemic index of soybeans could be beneficial to diabetics because of decreased atherosclerosis and normalization of blood glucose (Kurzer and Xu, 1997). Substituting animal protein with soy protein has been shown to normalize glomerular filtration rates by reducing kidney damage in diabetics with nephropathy. (Kurzer and Xu, 1997). It is believed that soy's estrogenic properties are responsible for its benefits in osteoporosis and menopause (Barnes et al., 1990). The structure of genistein (5,7,4'-trihydroxyisoflavone) differs from the basic flavonoid by the presence of the hydroxyl group on the 5' B ring (Wei et al., 1995) (**Figure 4**). It is estimated that a typical Asian diet contains from 20 to 80 mg/d of genistein (Aldercreut et al., 1993), whereas the typical Western diet contains about 24 mg/d of genistein (Wang and Murphy, 1996).

Epidemiological Studies: Quercetin and Genistein

A number of studies suggest that high fruit and vegetable consumption is associated with a decreased risk of human malignancies (WCR and AICR, 1997). However, most cohort studies have not been able to link flavonoid intake alone with a decreased incidence of cancer types (Arts et al., 2001; Goldbohm et al., 1995; Hertog et

al., 1993; Hertog et al., 1995). In the Zutphen Elderly Study, 805 men aged 65-84, had their diet assessed over a 5-year period. The intake of flavonols and flavones averaged about 26 mg/d, with tea, onions, and apples being the richest sources. There were no associations between flavonol and flavone intake and cancer of the stomach, colon, or lung (Hertog et al., 1993). The Zutphen Elderly Study was one of the cohorts of the Seven Countries cross-cultural study. This study investigated the relationships between diet, lifestyle, and disease over a period of 25 years in a population within the age range of 40-59 years. The intake of flavonols and flavones was compiled for each cohort. The average daily intake of flavonols and flavones ranged from 3 mg/d in a Finnish cohort to 70 mg/d in a Japanese cohort. The major dietary sources of flavonoids varied significantly between cohorts. Overall, there were no associations between cancer mortality and flavonoid intake (Hertog et al., 1995). The Netherlands study involved a 4.3-year follow-up of a cohort that included 58,279 men and 62,573 women aged 55-69 years and also reported no associations between flavonol/flavone intake and the incidence of stomach, colon, breast, or lung cancer (Goldbohm et al., 1995). In contrast, a Finnish study conducted over a 6 year period with 9,959 men, demonstrated that dietary intake of flavonoids was inversely associated with incidence of cancer at all sites combined. Cancer sites included lung, stomach, colorectum, urinary organs, prostate, and breast. This inverse relationship was usually observed in the case of lung cancer, with apples noted as a major dietary source of flavonoids, including quercetin. The total intake of flavonoids in the study population was 24 mg/d, which included an estimated intake of 3 mg/d of quercetin (Knekt et al., 1997).

In contrast to the cohort studies, several case control studies have indicated that flavonoids may decrease the incidence of cancer. Marchand et al. (2000) reported an inverse association between lung cancer risk and the consumption of onions, apples, or white grapefruit in a study of 582 lung cancer patients and matched controls. The total average flavonoid intake was 24 mg/d, including 9 mg/d quercetin. A study from Uraguay reported a significant inverse association of lung cancer incidence with increased consumption of carotenoid, glutathione, flavonoid, and vitamin E intakes in lung cancer patients (n=541 cases and n=540 controls); (De Stefani et al., 1999). Garcia-Closas et al. (1999) reported an inverse association between total flavonoid intake and the incidence of gastric cancer in cases versus matched controls.

Quercetin inhibits the proliferation of human cells from breast (Singhal, 1995), ovarian (Scambia, 1994), leukemic (Yoshida et al., 1992), and colon (Agullo et al., 1994) cancers. Several rodent studies have shown that quercetin administration can alter chemically-induced colon carcinogenesis. Rats supplemented with 0.45% quercetin had decreased incidence of aberrant crypt foci included with azozymethane (AOM), a colon carcinogen (Warren et al., 2003). Benzo{a}pyrene-induced nuclear damage in colonic epithelial cells was reduced with quercetin supplementation at 4% of the diet (Wargovich et al. 1985). In contrast, quercetin supplementation has also enhanced colon carcinogenesis. Periera et al. (1985) reported that 1.9% and 3.4% quercetin supplementation increased colonic adenocarcinomas in rats exposed to AOM. The mechanisms of how quercetin protected against or enhanced carcinogenesis are unclear.

There have been several epidemiological studies that have assessed the relationship between the consumption of soy products and cancer risk. In the studies

reviewed by Messina et al. (1994), 10 of 25 studies showed a statistically significant inverse relationship between soy intake and incidence of cancer at the following sites: rectal (1), stomach (5), breast (1), and lung cancer (3). In these studies, soy intake was measured using different methods, such as 3 day diet records or 24 hour recalls. Decreases in relative risk for cancer were observed with intakes of tofu at least 5 times per week (Severson et al., 1989), mean daily intake of 7 to14g of soybean products (You et al., 1988), and mean daily intake of at least 24 to 50 g of soy protein (Lee et al., 1991; Nomura et al., 1978). In contrast, fifteen studies showed no significant association between soy intake and the incidence of colon, breast, prostate, rectal, lung, stomach, and esophageal cancers (Messina et al., 1994). There are however, inconsistencies in the effect of soy products on incidence of cancer at different sites. Overall, the cohort, ecological, and case control studies suggest a protective effect of various soy products against different cancer types, but more research is needed in this area (Messina et al., 1994).

The epidemiological data has not been convincing regarding the relationship between flavonoid intake and cancer incidence, but there are clear associations between fruit and vegetable intake and numerous cancer types (Steinmetz and Potter, 1996). The lack of a clear association between protective effects and flavonoid intake may be due to the contribution of other components within fruits and vegetables that were not assessed in the epidemiological studies, or it may be that a combination of nutrients and phytochemicals is needed. In the studies that did show an association, it is difficult to credit the decreased cancer incidence to phytochemicals due to the lack of phytochemical biomarkers. Another problem is the possible inaccurate measurement of phytochemical

intake because most food frequency questionnaires are not designed to assess the consumption of phytochemicals.

Along with the epidemiological studies have observed an association between dietary soy consumption and decreased cancer incidence (Haenszel et al., 1972; Hirayama, 1982; Nagai et al., 1982; Severson et al., 1989), a number of *in vitro* and *in vivo* animal studies have demonstrated the ability of soy foods or the isolated isoflavones to alter the activity of factor(s) involved in cancer development (Barnes et al., 1990; Fitzsimmons et al., 1989; Fotsis et al., 1993; Higashi and Ogawara, 1992),. Several cell culture and animal studies however, have demonstrated a deleterious effect of the isoflavones in mammary tissue (Wang et al., 1995; Wang et al., 1996; Welshons et al., 1990), and several human studies have failed to demonstrate the protective anticancer effects of isoflavones and soy products (Horn-Ross et al., 2001; Kurzer and Xu, 1997; Mitchell, 2001). Many environmental and biological factors can modify the metabolism and bioactivity effects of soy products. This makes it challenging to isolate the possible factors and doses capable of producing either protective or damaging biological effects (Kurzer and Xu, 1997).

Quercetin Absorption and Metabolism

Achieving significant physiological levels of dietary flavonoids depends upon their ability to be absorbed and their interactions with target tissues. At this point there is little known about their net transport across the intestine, which metabolites are responsible for positive biological activities, and what concentrations are needed to elicit an effect. The most bioavailable forms of quercetin are believed to be the quercetin

glycosides found in onions, such as quercetin-4'-O-ß-D-glucoside and quercetin-3,4'-O-bis-ß-D-glucoside (Herrmann, 1988; Kiviranta et al., 1988). Their bioavailability has been demonstrated in several studies by Hollman et al (Hollman, 1997; Hollman et al., 1997; Hollman et al., 1996). Their studies suggest that the various types, or lack, of sugar moieties bound to quercetin have an effect on the rate and amount absorbed. However, an earlier study by Manach et al (Manach et al., 1995) had found similar concentrations of quercetin and rutin in the plasma and fecal contents of rats fed the same molar amounts of quercetin or rutin. These results would indicate that the sugar moitey present on rutin did not facilitate the absorption of quercetin into circulation over the aglycone quercetin. In contrast, another study by the same group (Manach et al., 1997), administered the same diets to rats that contained either aglycone quercetin or rutin and it was found that aglycone quercetin was absorbed more rapidly and in higher concentrations than quercetin from the rutin compound. At the current time, in humans it is accepted that quercetin glycosides, especially from onions, are absorbed more efficiently than the aglycone forms of quercetin (Hollman, 1997; Hollman and Katan, 1997; Hollman et al., 1997).

Quercetin is ubiquitously found in nature in various glycosylated forms, meaning quercetin is bound to various sugar moieties found within fruits and vegetables. In the early research on quercetin absorption it was assumed that glycosylated quercetin passed through the small intestine and into the colon for de-glycolysation by colon micro flora. Once quercetin was released from its sugar moiety the lipophilic aglycone could then passively diffuse into the cell and be absorbed (Manach et al., 1995; Manach et al., 1997). That theory however, was challenged when a study by Hollman et al. (1997) found that

human subjects with an ileostomy were able to absorb quercetin from foods such as onions, apples, and a supplemental quercetin rutinoside, the major quercetin compound found in tea. Since the glycosylated quercetin did not reach the large intestine of those subjects, it was thought that the small intestine did have a role in the absorption of the hydrophilic glycosylated quercetin through a carrier mechanism present along the brush border membrane of the small intestine (Hollman et al., 1997). The carrier is believed to be the sodium-dependent glucose transporter (SGLT1), which is able to actively transport the intact glycosylated quercetin into the enterocyte, to be de-glycosylated by ß-glucosidases within the small intestine (Wolffram et al., 2002) (**Figure 5**). The role of SGLT1 as a flavonoid transporter is controversial. The other theory of how the water-soluble glycosylated quercetin is absorbed by the small intestine is by the action of the ß-glucosidase, lactase phlorizin hydrolase (LPH). LPH is an extracellular enzyme found along the brush border membrane of the small intestine, and is primarily responsible for the hydrolysis of lactose (Day et al., 2000). A deficiency of this enzyme causes lactose intolerance (Auricchio et al., 1963). LPH has two hydrophobic domains, the lactase and phlorizin sites, which are capable of hydrolyzing flavonoid glycosides. The phlorizin site hydrolyses phlorizin to the aglycone phloretin and glucose (Leese and Semenza, 1973). This is important because phlorizin is closely related structurally to flavonoid glycosides, which could make it possible that LPH also can metabolize the flavonol glycosides and may be responsible for the hydrolysis of quercetin glycosides. Once LPH hydrolyses quercetin from its glycoside the aglycone quercetin is passively diffused into the enterocyte (Williamson et al., 2000) (**Figure 5**). The other ß-glucosidases identified in mammals are all found within the enterocyte. They include the cytosolic broad-

specificity enzymes, located in the liver, kidney and small intestine (Daniels et al., 1981), glucocerebrosidase (Hayes et al., 1996), and pyridoxine glucoside hydrolase (McMahon et al., 1997), which are also located in the small intestine. The ß-glucosidases have different affinities for the glycosides. This affects the rate and location of flavonoid absorption. Other transporters along the brush border are intestinal efflux transporters, such as multi-drug resistance-related proteins (MRP). MRPs have been shown to excrete phase II conjugates from the liver, intestine, and kidneys of humans and rats (Ayrton and Morgan, 2001). The MRPs located on the wall of the small intestine, in both humans and rats (Taipalensuu et al., 2001; Walle et al., 1999), can efflux the glucuronidated/sulphated flavonoid conjugates into the intestinal lumen or portal vein (Figure 4). Its action can contribute to enterohepatic and enteric recycling (Liu and Hu, 2002).

The first stage of metabolism is probably the de-glycosylation of the flavonoid glycoside, which is essential before further metabolism can take place. To date there have been few studies to show the presence of intact quercetin glycosides in the plasma of rats (Walgren et al., 2000) and humans (Aziz et al., 1998; Mauri et al., 1999; Paganga and Rice-Evans, 1997). In two of those studies they based their identification of the glycosides on their retention times on an HPLC column (Aziz et al., 1998; Paganga and Rice-Evans, 1997). However, the retention time of glycosides is very similar to that of the glucuronide conjugates, and confirmation of their identity can only be conducted using mass spectrometry. Mauri et al. (Mauri et al., 1999) did use mass spectrometry to identify the metabolites in human plasma and confirmed the presence of an intact glycoside of quercetin known as rutin. It is currently debated as to whether intact glycosides can be absorbed and further research is needed in this area.

As discussed previously, the glycoside needs to be hydrolyzed. The de-glycosylation by the ß-glucosidases could take place on the brush border (LPH), in the gut lumen, or inside of the enterocyte if SGLT1 actively transports the intact glycoside into the enterocyte (**Figure 5**). Based on their catalytic efficiency, it has been suggested that quercetin glycosides are hydrolyzed more effectively than the isoflavone glycosides (Day et al., 2000). If the glycoside is not hydrolyzed by ß-glucosidases within the small intestine, which is the case for quercetin-3-rutinoside (Gee et al., 1998), then it passes into the colon to be hydrolyzed by the micro flora. Once this occurs the aglycone can be absorbed into the circulation.

If the quercetin glycoside is hydrolyzed in the lumen of the small intestine, the aglycone will be absorbed into the enterocyte and the PI and PII enzymes can metabolize it there or it can be passively diffused into the liver to be metabolized. If the quercetin compound is ingested in the aglycone form, as is the case in this study, the flavonoid has the potential to be passively diffused into the small intestine or liver. Once within either of these organs the action of the PI and PII enzymes produce water-soluble methylated derivatives and conjugates of quercetin through methylation, sulfation (sulphates), or glucuronidation (glucuronides) (Monrad et al., 1998; Piskula and Terao, 1998) (**Figure 5**). Converting the fat-soluble compounds to water-soluble substances through conjugation is necessary for their excretion. Conjugated compounds are released into the systemic circulation from the enterocyte or the liver to eventually be excreted into urine or bile. If a compound is secreted into bile the compound can be excreted in feces or re-circulated with the bile via enterohepatic circulation. When the liver excretes the bile and conjugated compounds, these pass through the small intestine and colon. The conjugated

compounds may be de-conjugated by glucuronidases present in the intestine. This would

allow the released aglycone to be absorbed into the circulation. The circulation of

flavonoids may affect their bioactivity due to increased exposure time to tissues

(Coldham and Sauer, 2000). Alternatively, re-circulation may result in poor

bioavailability because the flavonoids circulate as conjugates, and the bioactive aglycone

may not be exposed to the appropriate tissue needed to produce a protective biological

effect (Chen et al., 2002). Enteric recycling has been found to have a greater impact on

the flavonoid bioavailability than enterohepatic circulation (Chen et al., 2002). Enteric

recycling occurs when the aglycones within the enterocyte are conjugated and then

effluxed back into the lumen of the gut where they can be deconjugated and re-absorbed.

Enteric recycling is an important factor when considering the bioavailability of

flavonoids, such as quercetin and genistein, that are primarily conjugated in the small

intestine because they may not be absorbed into systemic circulation and instead pass into

the colon to be excreted (Chen et al., 2002).

Plasma Concentration of Quercetin

The concentration of quercetin that reaches the plasma in humans and rats fed

different forms of quercetin has been studied extensively. The peak plasma concentration

of quercetin is usually reached in 40 minutes past ingestion in rats and humans (Conquer

et al., 1998; Hollman et al., 1997; Hollman et al., 1996). In animal studies, plasma

quercetin concentrations ranged from 45 µmol/L to 120 µmol/L (Manach et al., 1995;

Manach et al., 1996; Manach et al., 1997). In humans the concentration ranged from 0.1

µmol/L to 1.34 µmol/L with an average concentration of 0.7 µmol/L (Aziz et al., 1998;

Hollman, 1997; Hollman et al., 1997; McAnlis et al., 1999; O'Reilly et al., 2001). Possible reasons for the large discrepancy in the plasma concentrations between the rat and human studies is the large dietary amounts fed to the animals compared to human studies, and interspecies variability in absorption and metabolism. Also the methylated and conjugated quercetin metabolites were measured in rat studies whereas in humans only the conjugated metabolites were analyzed.

The concentrations of quercetin used to treat cells *in vitro* or the dietary amounts administered in animal studies that have demonstrated quercetin's ability to produce positive biological effects usually exceeded the average plasma quercetin concentrations typically seen in humans as well as dietary intake (Castillo et al., 1989; Deschner et al., 1991; Deschner et al., 1993; Husain et al., 1987; Takahama, 1984; Tzeng et al., 1991; Yoshida et al., 1990).

Genistein Absorption and Metabolism

Genistein is commonly found in food products in its glycosidic form. An example is genistin. Only soy foods that have undergone fermentation with bacterial cultures, such as miso, natto, and tempeh contain appreciable levels of the aglycone isoflavones (Murphy, 1982). At this point genistein glycosides have not been detected in plasma (Anlauer et al., 2000; Setchell, 2002). The bioavailability of aglycone genistein is poor (King et al., 1996; Setchell, 2002; Xu et al., 1994). In some cases (Setchell et al., 2001; Setchell, 2003) the glycosides have been found to be more bioavailable than their aglycone counterparts due to greater absorption, as measured in plasma, as well as delayed excretion. The majority of studies however, have found that genistein glycosides

need to be hydrolyzed to their aglycone form before they can be absorbed from the gut lumen and into circulation. Possible reasons for poor uptake of glycosides include very slow passive diffusion into the tissue, being poor substrates for glucose transporters, and possible efflux of compounds out of the intestinal wall and back into the lumen (Liu and Hu, 2002). Once in the small intestine genistein can be conjugated there, possibly by uridine diphosphate glucuronidases or sulfotransferases, or it may enter the portal vein to be conjugated in the liver (Anlauer et al., 2000; Chen et al., 2002; King et al., 1996; Sfakianos et al., 1997). The result of this extensive first-pass metabolism is the presence of conjugated isoflavones in the plasma that may not be as bioactive as the parent genistein compound. The metabolite 7-OH-glucuronic acid and 4'-OH-sulfate have been found to be the main genistein metabolites in rats and humans (King 96, King 98, Setchell 2001, Zhang). Peak plasma values of genistein have been observed in rats to occur at 2 and 7 hours post prandial due to enterohepatic circulation (Coldham and Sauer, 2000; Sfakianos et al., 1997; Yasuda et al., 1996). In humans, peak plasma concentrations of aglycones were reached after 4-7 hours in a study by Setchell et al. (Setchell et al., 2001).

Similar to quercetin, it was believed that the hydrolysis of genistein glycosides took place in the gut by bacterial glucosidases and once the aglycone was absorbed it was metabolized extensively in the liver by Phase II enzymes (King et al., 1996). However, a study by Bowey et al. (Bowey et al., 2002) contradicted this earlier theory of absorption. They observed substantial amounts of aglycone genistein in the urine of micro flora free rats fed a diet containing soy protein. This demonstrated that the glycosides within the soy protein were hydrolyzed by a mechanism other than colon glucosidases in order for

the aglycones to appear in the urine. Recent studies have found that extensive

metabolism of genistein takes place in the small intestine and liver by essentially the

same mechanisms as quercetin (Liu 2001) (**Figure 5**). Recently Coldham et al (Coldham

et al., 1999) identified metabolites of genistein as they appeared in the urine and caecal

contents of rats gavaged with aglycone genistein. They also identified metabolites within

the culture medium of hepatocytes that were incubated with aglycone genistein. This

data revealed the formation of 4-hydroxy-phenyl-2-propionic acid (Gm1), genistein

glucuronide (Gm2), dihydrogenistein glucuronide (Gm3), genistein sulfate (Gm4),

dihhydrogenistein (Gm 5), 6'-hydroxy-O-desmethylangolensin (Gm6), and 4-ethylphenol

(**Figure 6**). With the exception of 4-hydroxy-phenyl-2-propionic acid all of these

metabolites have been found in human urine (Joannou et al., 1995). It has been

demonstrated that dihydrogenistein is not only formed from genistein by mircoflora, but

genistein can be hydroxylated by cytochrome P450 enzymes to form dihyrogenistein

(Bowey et al., 2002). In the proposed pathway of genistein metabolism the colon micro

flora has a significant role in the formation of metabolites, and the metabolites of the liver

and small intestine result from the action of phase II enzymes (**Figure 6**). In a follow-up

study, the same group identified the absorption, metabolism, tissue distribution, and

excretion of genistein in rats gavaged with aglycone genistein. They identified the

primary plasma metabolite of genistein as genistein glucuronide. They also discovered

that the sulphotransferase enzymes were quickly saturated as evidenced by the

disappearance of genistein-sulfates and increased appearance of glucuronides in the

plasma. Sulphotransferases have a lower K_m compared to glucuronosyl transferases

(Pang, 1990). Thus it has been hypothesized that as the intake of isoflavones increases,

the bioavailability of aglycone genistein absorption may increase due to the conjugating enzymes being saturated and unable to conjugate the overwhelming amount of aglycone genistein present. (Coldham and Sauer, 2000). Anlauer et al. (Anlauer et al., 2000) may have observed enzyme saturation when they used an isolated rat small intestine perfusion model. As the aglycone genistein was perfused into the lumen the lumenal disappearance of genistein was higher than the vascular appearance rate. This may indicate that the basolateral glucuronide transporter possesses a higher affinity for geinistein glucuronide compared with the transporter at the brush border membrane therefore slowing the release of the conjugates from the enterocyte. However, the slow release of the conjugate did not affect the ratio of free genistein to conjugated genistein in the blood. This indicates that both free genistein and genistein glurcuronide may remain in intestinal tissue. Using a bile duct-cannulated rat model Sfakianos et al. (Sfakianos et al., 1997) also noticed that as the high doses of aglycone genistein were infused into the small intestine there was a higher rate of absorption compared to biliary excretion of conjugated genistein. The delay could have been due to saturation of phase II enzymes or hydrolysis of the glucuronide to aglycone genistein within the intestine, leaving the genistein to be slowly passively diffused into the portal vein. They also found that bypassing the small intestine and infusing the aglycone genistein into the portal vein resulted in aglycone genistein in peripheral circulation. This also suggests PII enzyme saturation in the liver. At this time the concentration of genistein needed to saturate the first pass effects of the small intestine and the liver is not known and the biological consequences of doing so are also unknown.

Plasma Concentrations of Genistein

While sulfate and glucuronide conjugates of genistein have been identified as the major metabolites in both humans and rats, little is known of their potential biological activities (Zhang et al., 2003). In humans fed various soy products the plasma concentration of total genistein (free + conjugated) ranged from 0.4 - 2.2 µmol/L (Setchell et al., 2001; Setchell, 2002; Setchell, 2003; Setchell et al., 2003; Zhang et al., 2003). These plasma concentrations are significantly less than the effective doses of 5 to 50 µmol/L needed to produce its anticancer effects *in vitro* (Birt et al., 2001; Kurzer and Xu, 1997; Yang et al., 2001). Inhibition of cancer cell proliferation has been observed with the hydroxylated and methylated genistein metabolites, suggesting that some isoflavone metabolites may have a role in producing a bioactive effect (Peterson et al., 1998). Further investigation of the potential benefits of metabolites would be logical since their manifestation in plasma has been shown to be significant.

Quercetin and Genistein as Mono/Bi-Functional Enzyme Inducers

Previous studies have demonstrated that the administration of the flavonoids quercetin and genistein can increase the activity of the PII enzymes. A few *in vivo* studies have measured enzyme activity after a single or multiple doses of diet administered flavonoids, usually at only one time point (Mikulcik and Fischer, 2001; Fischer and Fisher, 2000; Breinholt et al., 1999; Canivenc-Lavier, 1996; Wei et al., 1995; Siess, 1989; Brouard et al., 1988). Few studies have investigated whether PII enzyme activity will increase in a time and dose response manner.

The few animal studies that have investigated the ability of quercetin to influence the activity of the metabolizing enzymes have usually administered aglycone quercetin in

the diet (Mikulcik and Fischer, 2001; Fischer and Fisher, 2000; Canivenc-Lavier, 1996; Siess, 1989; Brouard et al., 1988). The dose, gender, and species of experimental animals have varied between studies. Presently there are no animal studies that have examined whether quercetin can mono-functionally induce PII enzyme activity in a dose response manner over time.

Previous feeding studies in our laboratory initially focused on determining whether doses of quercetin used to reduce colon tumor development in rats would significantly induce the activity of GST. In a 4 week feeding study male weanling Sprague Dawley (SD) rats (n=8 per diet group) were used to test the interaction between high dietary iron levels and quercetin in the kidney, liver, and the distal and proximal colon segments. Using a 3X3 factorial design, rats were fed a semi-purified diet containing 3 levels of iron (45, 650, and 1500 mg/kg diet) and 3 levels of quercetin 0, 1% (10 g/kg diet/d), and 2% (20 g/kg diet/d). Glutathione-s-transferase activity was measured in all of the tissues to determine if quercetin supplementation increased its activity. The results showed that iron supplementation did not significantly alter the activity of GST, but at 1% and 2% of the diet quercetin significantly increased the GST activity in the liver and colon. The activity of GST was not significantly increased in the kidney at any dose (Fischer and Fischer, 2000).

In a follow up study, low and high doses of quercetin were tested in order to determine the range at which quercetin is able to significantly induce PII enzyme activity. During a 6 week feeding study, twenty-four of the rats were fed a semi-purified diet with three levels of quercetin, 0%, 0.2% (2 g/kg diet), and 1% (10 g/kg diet). The levels of quercetin were beyond what could be reached by either supplementation or the human

diet. The liver, proximal and distal colon mucosa were removed for GST activity

analysis. Supplementation of 1% quercetin significantly increased colon GST activity,

whereas 0.2% quercetin showed a non-significant increase in colon GST activity

(Figures 7 and 8) (Mikulcik and Fischer, 2001).

These results indicated that further studies were necessary to determine the

minimal level of quercetin needed to significantly increase GST in the colon mucosa and

to examine whether a concentration of quercetin between 0.2% and 1% will increase GST

activity in the liver and the kidney. Also, these studies did not measure enzyme activity

after less than four weeks of feeding, nor was activity measured to determine whether it

was maintained over a period of time. These are important aspects to investigate because

of the high variability of peak enzyme activities. (Oesch and Arand, 1999).

Studies investigating the effect of genistein on enzyme activity have varied in

rodent species and strains, gender, length of study, administration route, dose, and form

of genistein (Ronis et al., 2001, Rowlands et al., 2001, Appelt and Reicks, 1999;

Breinholt et al., 1999; Appelt and Reicks 1997; Cai et al., 1997; Mirsalis 1993).

Currently, there are no *in vivo* dose and/or time-response studies to test the effect of

isolated genistein on GST activity**.** Appelt and Reicks (1997) measured GST and QR

activity after 7 and 14 days of treatment, in the liver, kidney, and colon of 7-8 week old

male SD rats. They were fed soy flour (SF) or soy protein isolate (SPI) amounting to

daily consumption of 0.0002% genistein (0.002 g genistein/ kg diet) and 0.0003% (0.003

g genistein/ kg diet), respectively. There were significant increases in liver and kidney

GST and colon QR at 14 days. Dietary source did not result in differences in enzyme

activities. In a follow-up study by the same research group (Appelt and Reicks, 1999) the

same enzymes and tissues were measured at 2 and 13 weeks using 9 week old female SD rats fed SPI containing low 0.00016% genistein (0.0016 g genistein/kg diet), medium 0.00088% genistein (0.0088 g genistein/kg diet), and high 0.00225% genistein (0.0225 g genistein/kg diet) doses in their daily food intake. The low/medium doses were comparable to the intake of regular human consumption of soy, whereas the high dose was two to three times higher than typically seen in an Asian diet. The results demonstrated that dietary soy isoflavone needed to be fed at the high dose to induce PII enzymes (Appelt and Reicks, 1999). Wei et al. (Wei et al., 1995) also found increased GST activity in the lung, liver, and kidney of female 6 week old SENCAR mice fed a diet, for 30 days, containing 50 ppm genistein (0.05 g genistein/kg diet).

Currently, there are few studies that have investigated the ability of the two flavonoids to mono-functionally induce the PII enzymes *in vivo*, and the current data is conflicting. An *in vitro* (Yannai et al., 1998) study demonstrated an overall effect of quercetin as a mono-functional inducer of a PII enzyme, whereas genistein was found to exhibit bi-functional and slight mono-functional potency. The mono or bi-functional induction by genistein appeared to depend on the concentration of genistein used to treat the cells. An *in vivo* study conducted by Breinholt et al. (1999) gavaged 8-9 week old female Wistar rats with 0.1 g/kg body weight of quercetin or genistein. Flavonoids were gavaged for 14 d and on day 14 the animals were gavaged with the carcinogen 2-amino-1methyl-6-phenylimidazo {4,5-b}pyridine (PhIP). After a 24 hour exposure the animals were sacrificed. They found that quercetin and genistein failed to induce liver or colon GST activity, decreased QR in liver and had no effect on colon QR activity. Neither flavonoid induced CYP1A1 activity in the liver (Breinholt et al., 1999). Other *in vivo*

studies that have measured both PI and PII enzymes have shown that quercetin does not

increase CYP1A1 or GST activity at various doses in the liver or small intestine

(Canivenc-Lavier et al., 1996; Siess et al., 1989; Brouard et al., 1988). However, the

doses used may not have been appropriate for the particular tissue analyzed in order to

increase GST activity. A study by Brouard et al, administered 1% quercetin (10 g/kg

diet) in the diet, which did not significantly increase PI or PII enzyme activity in the liver

or small intestine in a 14 day feeding study using male SPF Wistar rats (Brouard et al.,

1988). Similarly, Siess et al. (1989) and Canivenc-Lavier et al. (1996) found that

quercetin did not induce CYP1A1 or GST liver activities of male SPF Wistar rats fed a

diet containing 0.3% quercetin (3 g/kg diet) for 14 days.

Genistein's ability to mono-functionally decrease PI enzyme activity, and activate

various PII enzymes have been demonstrated when both enzyme systems have been

tested *in vivo* and *in vitro*. For example, genistein was able to inhibit CYP1A1 activity in

mouse Hepa 1c1c7 cells. The cells were first exposed to 1 nmol/L 2,3,7,8-

tetrachlorodibenzo-p-dioxin (TCCD) for 24h and then treated with several concentrations

of flavonoids for the next 48 hours. Fifty percent inhibition of CYP1A1 was reached at

24 umol/L genistein (Shertzer et al., 1999). A study by Mirsalis (Mirsalis et al., 1993)

demonstrated genistein's ability to be a mono-functional inducer *in vivo*. Six week old

male B6C3F1 mice fed soya bean flakes (SBF) for 30 days had increased hepatic GST

activity at 25% (0.2 g genistein/kg diet/d) and 2.5% SBF diet (0.02 g genistein/kg

diet/d), genistein significantly decreased CYP1A1 activity at all doses of SBF. Other *in

vivo* studies examined the effect of SPI on a PI enzyme without concurrently

investigating activity of a PII enzyme. The results illustrated that female SD rats fed SPI

had lower 7,12-dimethylbenz-{a}anthracene (DMBA) induced CYP1A1 hepatic activity compared with those fed whey or casein protein (Rowlands et al., 2001). A similar study found that the induction of liver CYP1A1 activity, by 3-methylcholanthrene (3-MC), was lower in male SD rats fed SPI compared to whey or casein diets (Ronis et al., 2001).

The conflicting results of the animal studies (**Table 1**) utilizing quercetin or genistein make it difficult to draw any definite conclusions concerning which doses and at what time increments the administration of genistein and quercetin will elicit an effect on the metabolizing enzymes. Therefore more research is warranted to determine at which doses, time points, and tissues the flavonoids are capable of inducing a mono-functional response of increasing PII enzymes. I tested the hypothesis that: the dietary administration of the flavonoids, genistein and quercetin, will mono-functionally increase the activity of the phase II detoxification enzymes glutathione -S- transferase, and quinone reductase in the liver, kidney, colon, and lungs of male Sprague-Dawley rats. The objectives of this study were: 1) to investigate if genistein and quercetin will mono-functionally induce the activity of the PII enzymes, GST and QR, without inducing the activity of the PI enzyme CYP1A1. 2) To determine if enzyme activity increases in a dose response manner. 3) To examine if change in enzyme activity is maintained over a 14 to 28 d time period.

Table 1

Previous Animal Studies Demonstrating the Effects of Quercetin and Genistein on the Metabolizing Enzymes

Author	Animals	Length of Study/Measure Enzyme Activity	Route of Adm.	Dose(based on 20 g diet/d)	Results
Mikulcik et al. 2001	male SD rats	6 wks	diet, aglycone qct	0, 0.2%, 1% (40, 200mg/d)	1% qct incr colon GST 0.2% incr trend in colon GST
Fischer et al. 2000	male SD rats	4 wks	diet, aglycone qct	0, 1%, 2% (200, 400mg/d)	1% and 2% qct incr liver and colon GST
Canivenc-Lavier et al. 1996	male wistar rats	1 wk acclimation then fed qct diet for 2 wks	diet, aglycone qct	0.3% (60mg/d)	no changes in GST or 1A1
Siess et al. 1989	Male SPF wistar rats	2 wks	diet, aglycone qct	0.3% (60mg/d)	no changes in GST or 1A1
Brouard et al. 1988	Male SPF wistar rats	acclimated for 2 wks then fed qct diet for 2 wks	diet, aglycone qct	1% (200mg/d)	no changes in GST or 1A1
Ronis et al. 2001	male SD rats	parents fed SPI and offspring were weaned to SPI at 32 d until 65 d Exposed to 3-MC for 17h then sacrificed	diet, SPI	~ 5.5 mg/d Gen	3-MC induced 1A1 activity was lower in liver of SPI fed rats compared to CAS and WHEY protein diets
Rowlands et al. 2001	female SD rats	parents fed SPI and offspring were weaned to SPI at 32 d until 48 d Exposed to DMBA for 24h then sacrificed	diet, SPI	~ 5.5 mg/d Gen	DMBA induced 1A1 activity was lower in liver of SPI fed rats

Author	Animals	Length of Study/Measure Enzyme Activity	Route of Administration	Dose(based on 20 g diet/d)	Results
Appelt et al. 1999	female SD rats	2 wks and 13 wks	diet, SPI	**lo:** 0.0002% (0.04 mg/d) **med:** 0.0009% (0.18 mg/d) **hi:** 0.002% (4 mg/d)	**2wk: med dose:** incr kid GST, incr colon QR, decr kid QR **hi dose:** incr liver GST, kid GST colon QR, decr kid QR **13wk: hi dose:** incr liver&colon GST, incr colon & kid QR
Breinholt et al 1999	female wistar rats	acclimated for 7 d then flavonoids were administered for 14 d PhIP was gavaged at 14 d	gavage, aglycone gen & qct	~20 mg/d (0.1g/kg bw)	qct and gen: no changes in GST or 1A1 activity, and decr in liver QR
Appelt et al. 1997	male SD rats	1 wk and 2 wks	SPI or SF	**SPI:** 0.0003% (0.06 mg/d) **SF:** 0.0002% (0.04 mg/d)	**1wk SPI:** incr colon QR **1wk SF:** incr liver GST, incr colon QR **2wk SPI:** incr liver GST, incr colon QR **2wk SF:** incr liver GST, incr kid GST, incr colon QR
Cai et al. 1997	female SENCAR mice	4 wks	diet, aglycone gen	**lo:** 0.0003% (0.06 mg/d) **hi:** 0.001% (0.2 mg/d)	**lo dose:** incr liver, kid, lung GST **hi dose:** no significant changes in GST activity
Mirsalis et al 1993	male B6C3F mice	30 d and 90 d	diet, SBF	0.8%(0.01mg/d) 2.5%(0.4mg/d) 8.0%(1.28mg/d) 25% (4mg/d)	**30d:** all doses decr 1A1, only 2.5% incr GST **90d:** incr trend in GST activity, no changes in 1A1 activity

*Abbreviations: Qct (quercetin), gen (gensitein), SPI (soy protein isolate), SBF (soya bean flakes), SF (soy flour), CAS (casein), 3-MC (3-methylcholanthrene), PhIP (2-amino-1methyl-6-phenylimidazo [4,5-b]pyridine), GST(Glutathione-s-transferase, QR(quinone Reductase) and 1A1 (cytochrome P450 1A1)

Figure 1

Bi-functional induction, by dioxin, through the xenobiotic response element (XRE).
Dioxin binds to the aryl hydrocarbon receptor (Ah)/heat shock protein 90 (hsp90)
complex. Dioxin disassociates from the hsp and binds to the aryl hydrocarbon receptor
nuclear transferase (ARNT) molecule. This goes into the nucleus and binds to the
XRE and synthesizes the mRNA for CYP1A1 enzyme. Reprinted with copyright
permission. *Guengerich, PF (1993) American Scientist. 81: 440-448.*
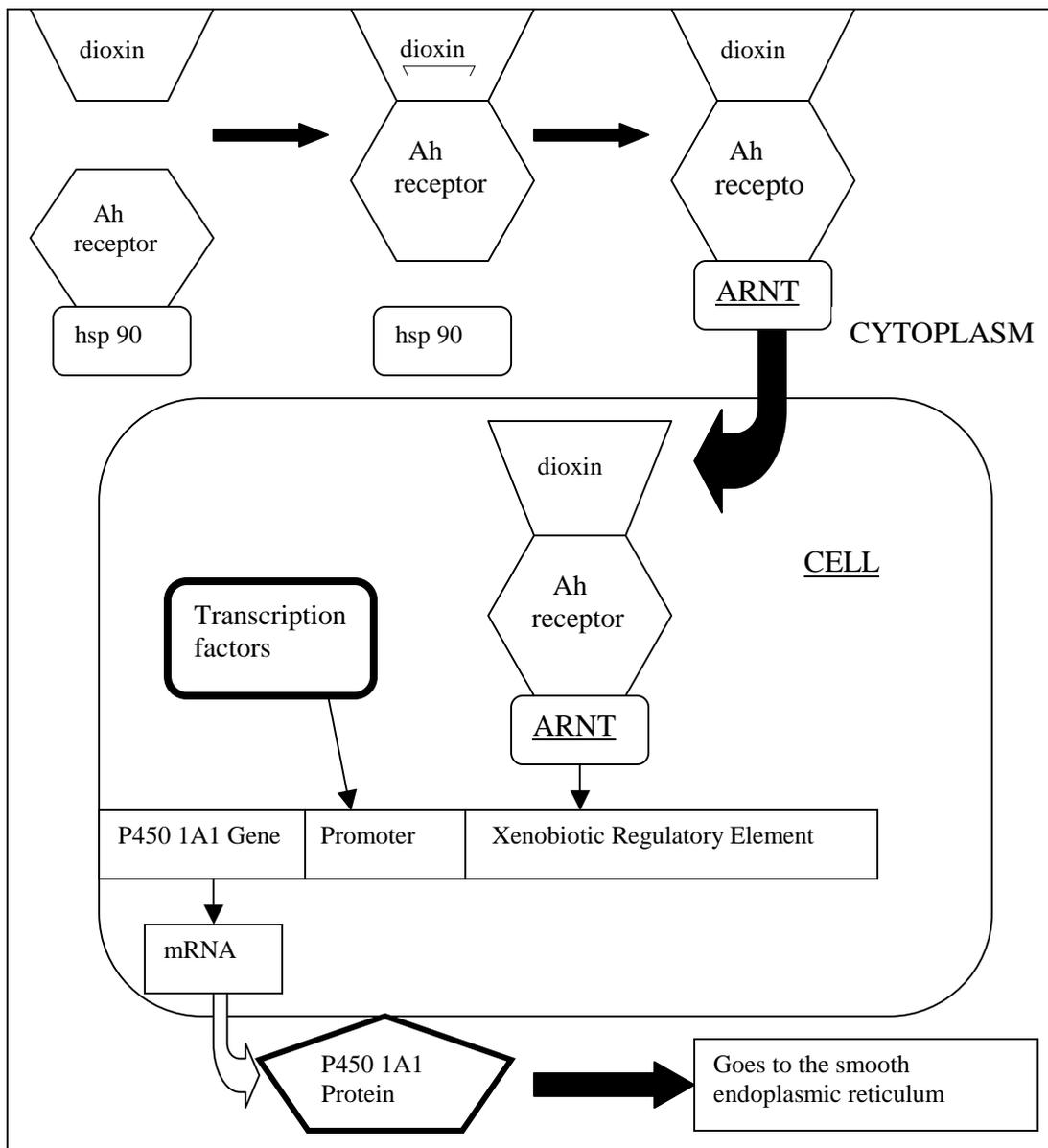
Figure 2

> The general structure of a flavonoid.
> Breinholt V, et al. (1999) Xenobiotica 29;12: 1228
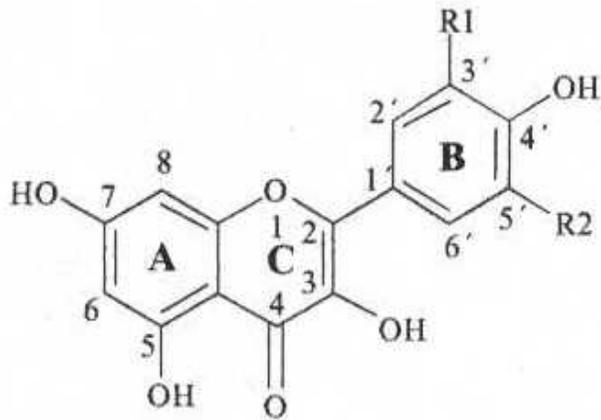
Figure 3

> The flavonol quercetin.
> *Breinholt V, et al. (1999) Xenobiotica 29;12: 1228*
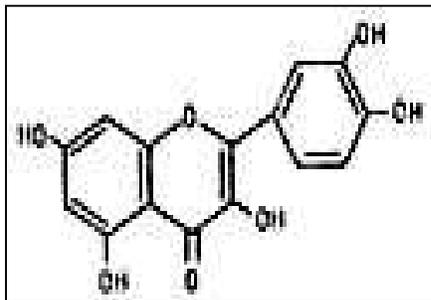
Figure 4

> The isoflavone genistein.
> *Breinholt V, et al. (1999) Xenobiotica 29;12: 1228*
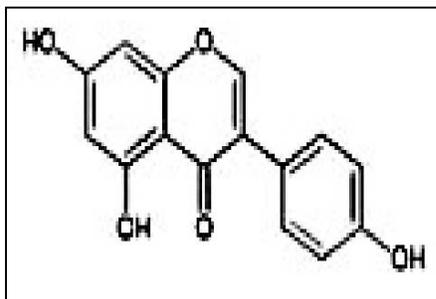
Figure 5
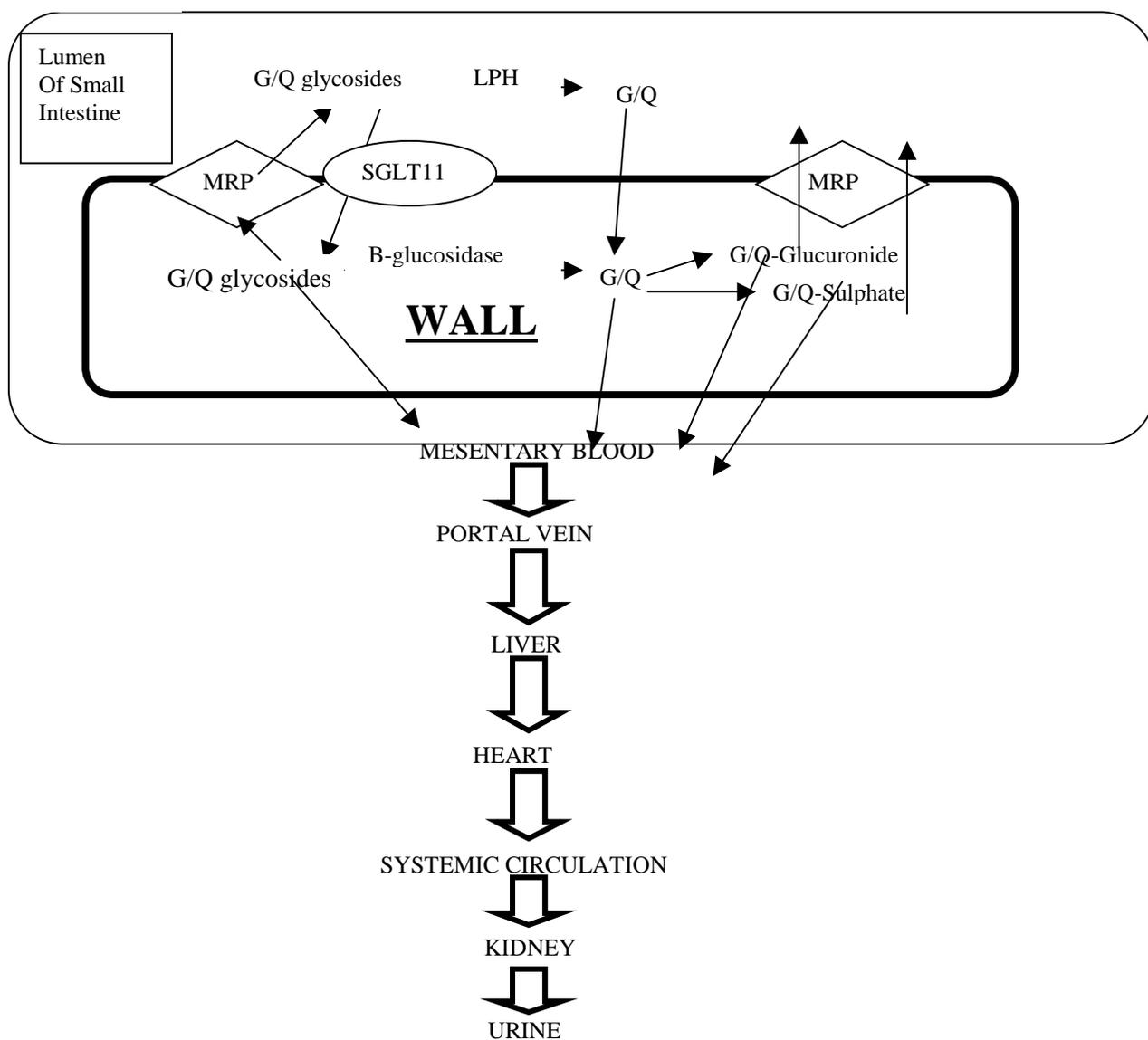
Absorption and metabolism of quercetin (Q) and genistein (G) into the lumen of the small intestine. The flavonoid glycosides can be transferred across the intestinal wall by sodium-dependent glucose transporter (SGLT1) or lactase phlorizin hydrolase (LPH) removes the glycoside and the aglycones passively diffuse into the intestinal wall. Once inside, the flavonoid glycosides can be hydrolyzed by B-glucosidases to the aglycone compounds. The aglycones may or may not be conjugated and can diffuse into mesentery blood. The conjugated or glycosylated forms can be expelled from the intestinal wall into the lumen or into the mesentery blood flow by multi drug resistant protein (MRP).*Liu, Y et al (2001) Drug Metabolism and Disposition. 30(4); 376. Reprinted with copyright permission.*

Figure 6

The proposed pathway of genistein biotransformation in the rat.
Reprinted with copyright permission.
*Coldham, NG et al (1999) The Journal of steroid biochemistry
and molecular biology. 70 (4-6); 169-84.*

Metabolites of The Cecum   Liver and Intestine Metabolites

Genistein-Glucuronide (Gm 2)

Genistein

Genistein-Sulfate (Gm 4)

Dihydrogenistein (Gm5)   Dihydrogenistein-Glucuronide (Gm 3)

6'hydroxy-O-desmethylangolensin (Gm 6)

4-hydroxyphenyl-2-propionic acid (Gm 1)

4-ethylphenol
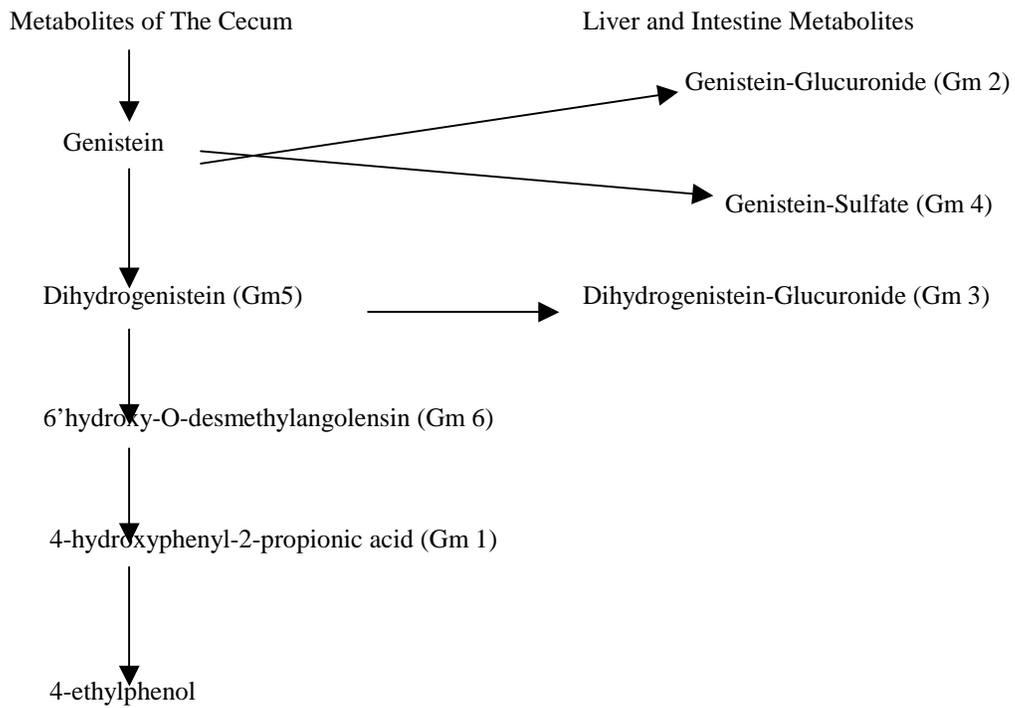
Figure 7

GST activity in distal colon of rats (n=8/group) fed 0%, 0.2%, or 1% quercetin. A column with different superscript letters differ significantly (p<0.05). *Mikulcik E, et al. (2001) The Journal of the American Dietetic Association.* Abstract;vol.101,
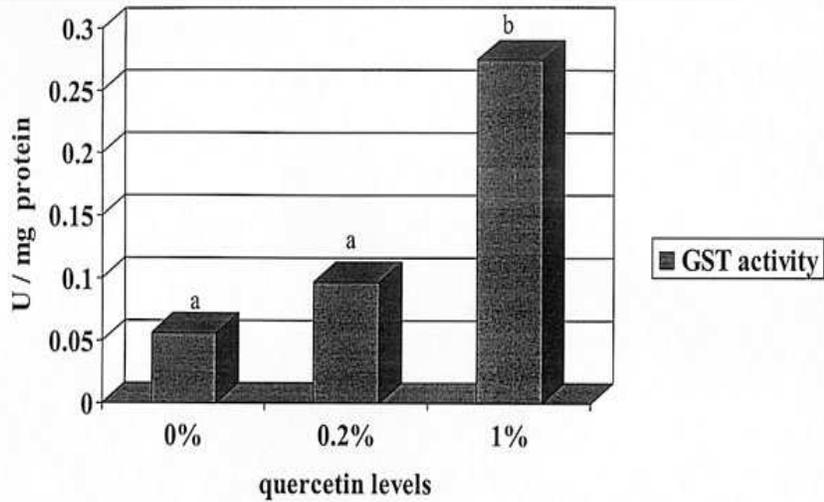
Figure 8

GST activity in proximal colon of rats (n=8/group) fed 0%, 0.2%, or 1% quercetin. A column with different superscript letters differ significantly (p<0.05).
Mikulcik E, et al. (2001) The Journal of the American Dietetic
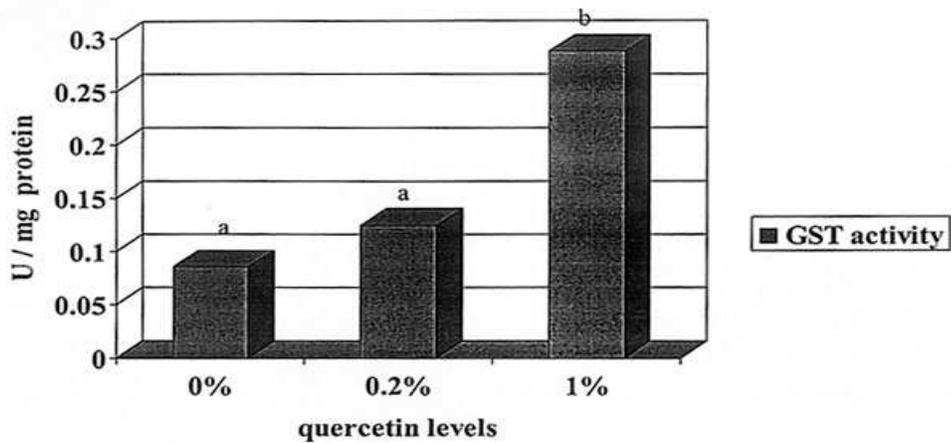
CHAPTER 3

EFFECTS OF THE PHYTOCHEMICALS QUERCETIN AND GENISTEIN ON

PHASE I AND PHASE II ENZYME ACTIVITIES[1]

Abstract

Quercetin and genistein are phytochemicals found in fruits and vegetables that may increase activities of anti-cancer Phase II enzymes. It was hypothesized that supplementation with these compounds would increase activities of the Phase II enzymes quinone reductase (QR), and glutathione-S-transferase (GST) in a dose response manner while decreasing or having no influence on the activity Phase I enzyme, CytochromeP4501A1 (CYP1A1). Enzyme activity was measured in liver, kidney, lung, and colon mucosa of male Sprague Dawley rats (n= 7-8/group) fed 0.3% (QC1), 0.6% (QC2), and 0.9% (QC3) quercetin or 0.0008% (GS1), 0.0012% (GS2), and 0.002% (GS3) genistein for 14 and 28 days. Significant changes (p<0.05) varied with compound, dose, organ, and time. There was a clear dose response increase in colon QR activity, at all doses of quercetin, whereas genistein had no effect on this enzyme. At 14 days, but not 28, QC2 and QC3 quercetin diets increased colon GST above control. The QC3 diet, and GS1 and GS2 doses increased liver GST above control at 14d. A dose response decrease in CYP1A1 occurred in the colon of 14 day quercetin treated groups (p= 0.003), while genistein had no effect. CYP1A1 activity was not significantly increased in any tissue measured, which suggests that these flavonoids can increase Phase II enzymes while either decreasing or not changing Phase I activity. The variability in enzyme induction between the compounds may be related to differences in flavonoid structure or dose.

<u>Introduction</u>

High intakes of fruits and vegetables are associated with reduced risk of cancers of the oral cavity, esophagus, stomach, colon and lung (WCR and AICR, 1997). Vegetable and fruit intake is encouraged due to the potential chemoprotective actions of the vitamins, minerals, and phytochemicals in these foods. *In vitro* and *in vivo* studies have demonstrated that phytochemicals are capable of inhibiting various stages of the cancer process (Wattenberg, 1992). Phytochemicals can be found in a variety of plant sources such as vegetables, fruits, nuts, seeds, cereals, and legumes (Kuhnau, 1976), and in beverages like wine, beer, cider, tea, and cocoa (Havsteen, 1983). A class of phytochemicals, known as polyphenols, can be divided into 10 different classes depending on their basic chemical structure (Bravo, 1998). The bulk of polyphenols fall into the widely distributed flavonoid class. Two important flavonoid classes abundantly found in the food supply are flavonols and isoflavones. A flavonol that is commonly found in fruits and vegetables, particularly apples and onions, is quercetin, whereas the isoflavone genistein is amply found in legumes, particularly soybeans. The estimated average consumption of the common flavonoids, quercetin, kaempferol, myricetin, apigenin, and luteolin, in the Western diet is 23 mg/d (Hertog et al., 1993). Quercetin intake is estimated to be around 16 mg/day (Hertog et al., 1993).

Quercetin may exert its protective effects through many different mechanisms. Quercetin inhibits the proliferation of human cells from breast (Singhal, 1995), ovarian (Scambia, 1994), leukemic (Yoshida et al., 1992), and colon (Agullo et al., 1994) cancers. It also acts as an antioxidant (Rice-Evans et al., 1996), has anti-inflammatory activities, and may modulate the immune system (Havsteen, 1983). It also influences the

activity of the Phase I (PI) and Phase II (PII) enzymes which metabolize carcinogens (Fischer and Fisher, 2000; Mikulcik and Fischer, 2001; Yannai et al., 1998). Several rodent studies have shown that quercetin administration can alter chemically-induced colon carcinogenesis. Rats supplemented with 0.45% quercetin had decreased incidence of aberrant crypt foci induced with azozymethane (AOM), a colon carcinogen (Warren et al., 2003). Benzo{a}pyrene-induced nuclear damage in colonic epithelial cells was reduced with quercetin supplementation at 4% of the diet (Wargovich et al. 1985). In contrast, quercetin supplementation has also enhanced colon carcinogenesis. Periera et al. (1985) reported that 1.9% and 3.4% quercetin supplementation increased colonic adenocarcinomas in rats exposed to AOM. The mechanisms of how quercetin protected against or enhanced carcinogenesis are unclear.

Soy has been studied extensively because of the health benefits associated with the phytoestrogenic properties of its two major isoflavonoids, genistein and daidzein. It is estimated that a typical Asian diet includes 20-80 mg of genistein per day (Aldercreut et al., 1993), whereas the typical Western diet contains about 24 mg of genistein per day (Wang and Murphy, 1996). Soy has demonstrated anti-cancer effects for breast, prostate, endometruim and colon cancers (Setchell, 1998). Like quercetin, it has also been shown to alter the activity of the PII xenobiotic metabolizing enzymes (Appelt and Reicks, 1997; Appelt and Reicks, 1999; Breinholt et al., 1999; Mirsalis et al., 1993; Wei et al., 1995), which may protect against cellular damage (Oesch and Arand, 1999).

PI and PII enzymes are enzyme systems that are responsible for the removal of xenobiotics. Xenobiotics are generally lipophilic compounds that are found in the environment (Klaassen et al., 1986). Their lipophilic solubility allows them to easily

diffuse into tissues.   To be excreted through urine, bile, or exhaled, the compound needs

to be converted into a water-soluble substance (Klaassen et al., 1986). Detoxifying or

bioactivating the xenobiotic transforms the fat soluble xenobiotic to a hydrophilic

constituent. PII enzymes detoxify by conjugating non-reactive water-soluble components

to the lipophilic compound resulting in its harmless removal from the organism. Activity

of PI enzymes often results in bioactivation, and the formation of reactive electrophilic

compounds that bind to macromolecules, such as DNA (Klaassen et al., 1986). The PII

enzymes prevent binding of the reactive compounds by conjugating the destructive

products before they inflict damage. Therefore higher activities of PII enzymes

compared to PI enzymes is critical in preventing cellular damage, and is theoretically

protective against cancer. This can be accomplished by mono-functionally inducing PII

enzyme activity. Enzymes can be mono or bi-functionally induced, meaning there is

either an increase of only PII enzymes or both enzyme systems, respectively. Quercetin

(Fischer and Fisher, 2000; Mikulcik and Fischer, 2001; Yannai et al., 1998) and genistein

(Appelt and Reicks, 1997; Appelt and Reicks, 1999; Breinholt et al., 1999; Mirsalis et al.,

1993; Wei et al., 1995) have both demonstrated their ability to increase PII enzyme

activity, although their mechanism of action is not well understood. Several *in vivo*

studies have measured only PII enzyme activity at one time point after a single dose of

diet-administered flavonoids (Brouard et al., 1988; Canivenc-Lavier, 1996; Siess, 1989).

The studies that have tested the dose response capacity of the flavonoids either measured

phase II activity only (Appelt and Reicks, 1997; Appelt and Reicks, 1999; Cai and Wei,

1996; Fischer and Fisher, 2000; Mikulcik and Fischer, 2001), tested its mono-functional

abilities in a whole food (Mirsalis et al., 1993), or in aglycone form with a carcinogen

(Breinholt et al., 1999). To our knowledge there has not been an *in vivo* study done to

investigate if the aglycones quercetin and genistein are able to mono-functionally induce

PII enzymes in a dose and time response manner.

I tested the hypothesis that the dietary administration of the aglycone flavonoids,

genistein and quercetin, would mono-functionally increase and maintain the activity of

the PII detoxification enzymes glutathione -S- transferase (GST), and quinone reductase

(QR) over a period of time in the liver, kidney, colon, and lungs of male Sprague-Dawley

rats. The objectives of this study were: 1) to investigate if genistein and quercetin could

mono-functionally induce the activity of the PII enzymes, GST and QR, without inducing

the activity of the PI enzyme CYP1A1. 2) To determine if enzyme activity increases in a

dose response manner.   3) To examine if change in enzyme activity is maintained over a

14 to 28 d time period.

Methods

Animals, diet, and experimental design

Male Sprague Dawley (SD) rats, (n= 112; initial weight 55-90 g, from Harlan

Indianapolis, IN). SD rats were housed individually in stainless steel wire-bottomed

cages in a temperature (21°C)-, humidity-, and light- (12 hour light:dark cycle),

controlled environment. All animal procedures were approved by the University of

Georgia Institutional Animal Care and Use Committee. Upon arrival, rats were

acclimated for 24 hours, and divided into weight matched groups and were assigned ed to

one of seven different weight-matched treatment groups (n=16 per group; mean group

weights: 70-74 g). Rats were fed ad libitum an AIN-93 diet supplemented with low,

medium, and high doses of either aglycone quercetin (Sigma Chemical Company, St.Louis, MO, USA) or genistein (Toronto Research Chemicals, ON, Canada) (**Table 2**). The levels of quercetin used were 0, 0.3% (QC1) (300 mg/100 g diet, 3.0 mg/ kg bw; 3.0 mg/kg bw), 0.6% (QC2) (600 mg/100 g diet, 6 mg/ kg bw; 6 mg/kg bw), 0.9% (QC3) (900 mg/100 g diet, 9.0 mg/kg bw; 9 mg/kg bw). The levels of genistein used were 0.0008% (GS1) (0.8 mg/100 g diet, 0.008 mg/kg bw; 0.007 mg/kg bw), 0.0012% (GS2) (1.2 mg/100 g diet, 0.009 mg/ kg bw; 0.009 mg/kg bw), and 0.002% (GS3) (2 mg/100 g diet, 0.014 mg/ kg bw; 0.018 mg/ kg bw). The amount of the flavonoids per kg of body weight were calculated using the mean initial and final body weights of 74 to 233 g, respectively. Tissues were collected after 14 (n= 8 per dietary treatment) and 28 day (n= 8 per dietary treatment) of feeding. Rats had free access to water throughout the study. Feeding of the experimental diets was initiated according to a staggered feeding schedule that coincided with the date of sacrifice to ensure that all rats were fed their diet for either 14 or 28 day. Body weight was measured weekly and food intake was measured at four different time points for three consecutive days. Food intake was measured on days 2, 3, 4; days 9, 10, 11; days 16, 17, 18; and days 23, 24, 25. Fresh food was added daily and diet was stored at -20°C until use.

Tissue Collection

The animals were fasted overnight prior to sacrifice. Rats were anesthetized with a 3:2:1 (vol/vol/vol) of ketamine:acepromazine:xylazine (0.8 mL/kg body weight). The liver, colon, kidneys, and lungs were removed following whole body perfusion. An incision was made from the abdomen to the chest area, and the skin was folded back from

the rib cage in order to make two cuts through the ribs. A canula was placed through the apex of the left ventricle of the heart and inserted into the aorta. Ice-cold heparinized saline was pumped through the animal for about ten minutes to remove the blood. The liver, lungs, and kidneys were excised, blotted dry and weighed. The collection of colon mucosa was done by first excising the colon and rinsing it with saline. The colon was placed on an ice-cold glass plate, cut open lengthwise, and scraped gently with an ice-cold microscope slide to collect mucosa. All tissues were dipped into liquid nitrogen and then stored at -80°C until analyzed.

Tissue Analysis

The tissues were homogenized at 4°C in phosphate buffer (pH 7.0, 0.1 mol/L potassium phosphate) using a hand held (Omni International, Tampa, FL) homogenizer. To obtain the cytosol fraction the homogenized tissues were centrifuged in a J2-HS Beckmen Centrifuge (Beckman Instruments Inc., Fullerton, CA) for 20 minutes at 4°C and 10,000 X G. The supernate was saved and transferred to a polycarbonyl centrifuge tube and then centrifuged again in a Beckmen Optima LE-80K Ultracentrifuge (Beckman Instruments Inc., Fullerton, CA) for 1.16 hours, at 4°C, 100,000 X G. Supernate was removed, which was the cytosol, and stored at -80°C. The remaining microsomal pellet was re-suspended in phosphate buffer. A glass-stirring rod was first used to re-suspend pellet and then a pipette was used to further mix the microsomal homogenate. The microsomes were stored at -80°C.

GST activity was measured in the cytosol with a spectrophotometer (Beckman DU 650, Beckman Instruments Inc., Fullerton, CA) using methods described by Habig et

al. (1974) with 10 mmol/L 1-chloro-2,4-di-nitrobenzene (CDNB) as the substrate.

Briefly, 0.2 to 30 mg tissue cytosol protein was incubated in the reaction mixture. The

reaction mixture, reached a final volume of 1 mL, and included 0.1 mol/L potassium

phosphate buffer (pH 6.5), 6.2 mmol/L glutathione, and 10 mmol/L CDNB. The reaction

was initiated upon addition of CDNB. The velocity of formation of S-(2-chloro-4-

nitrophenyl) glutathione was measured for 3 minutes, with readings taken every 30

seconds, at 340 nm, at 25°C. One unit of enzyme activity = 1 nmole conjugate

formed/minute/mg protein. The molar extinction coefficient for CDNB, at 340 nm, is 9.6

nmol/mL.

QR activity was also measured in the cytosol using methods described by Kore

et al. (1993) with 12 mmol/L 2,6-dichloroindophenol as the substrate (DPIP). QR

activity was measured in triplicate with a spectophotometer (Beckman DU 650, Beckman

Instruments Inc., Fullerton, CA). The total volume of the cuvette reached 1 mL. The

contents included 0.06 to 0.28 mg tissue cytosol protein incubated with 25 mmol/L Tris-

HCl (pH 7.4), 0.7 mg bovine serum albumin, 1% Tween 20, 5 µmol/L FAD, and 0.2

mmol/L NADPH and 0 or 10 µmol/L dicoumarol at 25˚C. DPIP (40 µmol/L) was added

to initiate the reaction. The reduction of DPIP was measured at 600 nm, for 3 minutes,

with readings taken very 15 seconds. The dicoumarol sensitive portion of the activity

was taken as a measure of the quinone reductase activity. The molar extinction

coefficient for DPIP, at 600 nm, is 2.1 nmol/mL

CYP1A1 activity was measured in the microsomes using methods described by

Chang et al. (1998) with 7-O-Ethoxyresorufin as the substrate. The activity of the

CYP1A1 enzyme was measured in duplicate using a spectroflurometer (Model RF-5301,

Shimadzu, Columbia, MD). A volume of 25 µL microsomal protein (dilute as needed) was added to cuvette, along with reaction mixture, for a total volume of 3 mL. The mixture contained 0.1 mol/L potassium phosphate buffer (pH 7.4), 50 mmol/L NADPH, and 0.75 mmol/L ethoxyresorufin. The sample, buffer, and ethoxyresorufin were incubated for 4 minutes, at 37˚C in a water bath while shaking at low speed. At the end of 4 minutes 100 µL NADPH was added to initiate the reaction. The formation of resorufin is read at an excitation wavelength of 550 nm and emission wavelength of 581 nm, for 2.3 minutes, with reading taken every 10 seconds, at 20ºC. Kidney CYP1A1 activity could not be measured due to low enzyme activity. Protein concentration of the microsomes and cytosol of each tissue was measured using the method of Lowry et al. (1951). Data were expressed as U/mg protein.

Statistics

Treatment means, standard error of the mean, analysis of variance (ANOVA), and least significant difference tests were determined using the statistical package SAS (version 6,10, SAS Institute, Cary, NC). The overall effects and interactions between the flavonoid dose and length of time were determined using 4X2 ANOVA. When it was determined that there were no time effects, a one way ANOVA was used to assess the effect of each flavonoid on enzyme activity separatedly at 14 and 28 days. Differences among treatment groups were considered significant at $p < 0.05$. Fisher's least significance difference test was used to assess the difference between means for dietary treatment groups.

Results

Food Intake, Body and Organ Weights

During the first week there was a significant decrease, of about 14%, in the consumption of all of the experimental diets compared to the control. This may have been due to the rats acclimating to the taste of the experimental diet (**Table 3**). There was no significant difference in body weight among treatment groups throughout the study (**Table 4**). The only significant elevation in liver to body weight ratio was found in rats fed 0.3% quercetin at the 14 day time point (p=0.02) (**Table 5**). It is not clear why the liver weights were increased in the majority of the animals within this treatment group. In the 28 day control group, analysis was not conducted on the organs of one rat within this group because black spots were observed on the liver of this animal. The organs of the remaining rats appeared normal. During the third week of the study one rat within the moderate dose of quercetin treatment group died. The cause was unknown, but all other rats in the study appeared normal.

Enzyme Activity

There was a dose response relationship shown between flavonoid dose and the activity of two of the enzymes measured in the colon mucosa. This occurred within the colon mucosa of the quercetin treated animals after 14 day of feeding. There was a dose response relationship between quercetin dose and an increase in QR activity as well as a decrease in CYP activity. In general, enzyme activity fluctuated depending on dose, flavonoid length of feeding, enzyme and organ (**Tables 6-11**).

Glutatione-S-Transferase Activity

At 14 days both quercetin (p=0.0006) and genistein (p=0.015) altered liver GST activity. Post-hoc tests showed that there was a significant increase in GST activity in the liver within QC3, GS1, and GS2 treatment groups compared to control (**Table 6**; **Figure 17**). Mean GST enzyme activity decreased slightly from 14 to 28 day in quercetin treated rats (p=0.01) and increased in activity within genistein treated rats (p=0.01), but this decrease was not consistent across treatment groups. After 14 days of treatment there was an increase in GST activity in rats consuming at least 0.6% quercetin, but by 28 day, when GST activity tended to be higher (p=0.06), there were no longer significant differences among quercetin treated groups (**Table 6**). Genistein had no significant effect on GST activity in colon mucosa. There were no increases or decrease in lung GST activity at 28 days, compared to controls, due to flavonoid consumption (**Table 7**). In the kidney,

after 28 days, rats fed 0.0008% (GS1) had a significantly higher level of GST activity than control or rats fed higher levels of genistein (p<0.01) (**Table 7**). The quercetin treated rats had a slight, but significant, increase in kidney GST activity at 28 days compared to 14 days (p=0.004), while mean kidney GST activity for genistein treated rats was significantly lower at 28 days than at 14 days (p=0.02).

Quinone Reductase Activity

Rats fed 0.9% quercetin had significantly higher liver QR activity, compared to controls, at both 14 and 28 days of feeding (**Table 8**; **Figures 11 and 12**). In contrast, rats fed genistein at all levels had lower liver QR activity than control rats (p=0.007),

although this occurred after 14 days of treatment. Mean QR activity was found to decrease over time from 14 to 28 days in both the liver and colon mucosa. Quercetin treated rats had a strong dose dependent increase in colon mucosa QR activity that resulted in up to 4-fold increases in activity (p=0.0001) after 14 days of treatment (**Figure 9**), and about a 2-fold increase in activity after 28 days of treatment (p=0.0002; **Figure 10**), compared to controls. However, genistein did not affect colon QR activity. There were no significant changes in lung QR activity as a result of quercetin treatment, but genistein treatment reduced QR activity after 14 (p=0.001) and 28 days of treatment (p=0.015; **Table 9**; **Figure 14**). Both quercetin and genistein decreased kidney QR activity after 14 days of treatment (**Figure 15**), but by 28 days of treatment, differences in QR activity between control and treated rats were no longer apparent (**Table 9**). In the kidney 0.9% QC increased QR activity, and all doses of GS decreased QR at 14 days (**Table 9**).

Cytochrome P4501A1 Activity

There were no significant increases in CYP1A1 activity within the liver, colon mucosa, or lungs of animals treated with either flavonoid, and genistein did not decrease CYP1A1 activity in any tissue (**Tables 10 and 11**). Quercetin however did inhibit enzyme activity in colon mucosa and lung. There was a decrease in CYP1A1 activity in the colon mucosa of rats treated with quercetin after 14 and 28 days of treatment (**Figures 18 and 19**). In contrast, in the lung, after 14, but not 28 days of treatment, 0.9% quercetin slightly increased the level of enzyme activity (**Table 10**).

Discussion

The purpose of this experiment was to investigate whether the flavonoids, quercetin and genistein, were capable of increasing the activity of the PII enzymes, GST and QR, while either decreasing or having no impact on the activity of the PI enzyme, CYP1A1. Enzyme activity was measured in liver, lung, colon, and kidney at two different time points to determine whether enzyme activity was maintained over time from 14 to 28 day. Based on a rat's typical intake of 20 g of diet/d the experimental doses of quercetin provided about 60 (0.3%), 120 (0.6%), or 180 (0.9) mg/d of quercetin while genistein treatment contributed 0.16 (0.0008%), 0.20 (0.001%), or 0.40 (0.002%) mg/d of genistein. Both quercetin and genistein were found to be mono-functional PII enzyme inducers, although in some cases the flavonoids also caused decreases in PII enzyme activity in various tissues. Other than the dose response changes in QR and CYP1A1 activity in the colon of quercetin treated animals, there were no other significant patterns of enzyme modulation. The effect of genistein and quercetin on enzyme activity varied depending on dose, compound, tissue, and length of feeding.

Liver GST activity was only increased at the highest dose of QC. Similarly, a study by Fischer and Fisher (2000) reported an increase in liver GST after 4 weeks of feeding rats 1% and 2% quercetin diets. This is in contrast to studies by Mikulcik and Fischer (2001) and Brouard et al. (1988) that used 1% dietary concentrations of quercetin in 6 and 2 week feeding studies, respectively, and saw no changes in activity. Other studies in which quercetin was fed at 0.3% of the diet to rats have not shown an impact on liver GST activity (Canivenc-Lavier et al., 1996; Siess et al., 1989), which is in agreement with our results. The consistent lack of enzyme induction at 0.3% quercetin indicates that this dose is not adequate to increase liver GST activity.

The consumption of a diet with 0.0008% and 0.0012% of genistein, but not 0.002%, was associated with increased liver GST activity after 14 days. Similar to these results, induction of liver GST by lower, but not high, doses of genistein has also been reported by others (Breinholt et al., 1999; Appelt and Reicks, 1997; Cai et al., 1997; Mirsalis et al., 1993). In contrast, soy protein isolate containing 4 mg of genistein per day was associated with higher liver GST activity after 2 and 13 weeks of treatment (Appelt and Reicks, 1999). These studies have demonstrated that the effect of genistein on liver GST activity is quite variable with time and dose. As with quercetin there was no set pattern of GST being induced after a certain length of exposure time. GST activity was induced anywhere from 2 to 13 weeks after treatment began. A possible pattern seen in the current study as well as in past studies, was that the lower doses of genistein (0.0002% and 0.0003%) (Appelt and Reicks, 1997; Cai et al., 1997) were able to increase activity, while higher doses (1% and 25%) (Breinholt et al., 1999; Mirsalis et al., 1993) did not. It is possible that the lower doses of genistein induced GST activity whereas the higher doses may have induced the activity of an enzyme other than GST. This is not known for certain because we did not measure PII enzymes other than GST and QR.

The colon mucosa may be exposed to high concentrations of unabsorbed flavonoids, which may account for the positive effects seen on PII enzymes in this study. Colon GST activity was enhanced by quercetin doses of at least 0.6% and 0.9% of the diet. These results agree with those of Mikulcik and Fischer (2000), which showed significantly increased GST activity with quercetin supplemention at 1% of the diet. The consistent effects on colon mucosa by quercetin may be explained by the poor bioavailability of aglycone quercetin in the small intestine (Hollman et al., 1997;

Hollman et al., 1996). Alternatively the absorptive capacity of the small intestine may have been saturated at higher doses, allowing more to pass into the colon where some absorption of quercetin continues, thus stimulating enzyme activity. Genistein did not increase colon GST activity, similarly to results of others (Appelt and Reicks, 1997 and 1999) who fed rats soy flour or soy protein isolate with low concentrations of genistein. Genistein's inability to increase GST colon activity may have occurred because genistein was not absorbed at the level of the colon, it was not present in high enough concentrations to induce activity, it never reached the colon, it was conjugated by other PII enzymes, or it was converted into a metabolite that was not a substrate for GST.

In the liver, only the highest level of quercetin was able to increase QR activity after 14 and 28 days. In contrast, Breinholt et al. (1999) showed a decrease in liver QR activity after rats were gavaged for 14 days with a 1% dose of quercetin. Their decrease in activity, however, may have resulted from a simultaneous administration of a carcinogen. In our study, all doses of genistein significantly decreased liver QR activity after 2 weeks, while Appelt and Reicks (1997 and 1999) found significant and non-significant increases in liver QR activity in rats fed soy protein isolate containing similar concentrations of genistein used in this study. This suggests that the isolated isoflavone may need the matrix of a soy product to prevent a decrease in activity. Breinholt et al. (1999) also showed a decrease in liver QR activity after rats were gavaged with a dose of genistein much higher than used in the current study.

Quercetin supplementation caused a dramatic, dose related response increase in colon mucosa QR activity. Breinholt et al. (1999) reported one of the only other *in vivo* studies that tested quercetin's effect on colon QR activity, and they found that quercetin

had no effect on colon QR activity. An important difference between the studies is that Breinholt et al.(1999) administered the carcinogen, 2-amino-1 methyl-6-phenylimidazo{4,5-b}pyridine, on day 14 and then measured tissue enzyme activity after d 24 hour exposure to the carcinogen. The acute exposure of the colon mucosa to PhIP may have saturated the QR enzyme. An explanation of why we saw such a large increase in activity within the colon mucosa compared to other tissues could be because the mucosa was exposed to higher concentrations of quercetin for a longer period of time than the other tissues. A study by Manach et al. (1997) provides evidence to support this explanation. They administered 0.2% aglycone quercetin (~ 40 mg/d), to male Wistar rats for either a single meal (non-adapted rats) or for 10 d (adapted rats). The concentration of quercetin was measured in the plasma and cecal contents. They observed that in the adapted animals the concentration of quercetin metabolites was higher than the single meal rats, but plasma metabolites, in the adapted rats, did not increase over a 24 hour period. In contrast, the single meal rats had a progressive accumulation of metabolites in the plasma until it leveled out by 24 hours. The lack of appearance of quercetin metabolites in the plasma of adapted rats suggested that, as with some micronutrients, the presence of a steady plasma concentration of a compound might depress the efficiency of intestinal absorption, thereby increasing the amount and time that quercetin spends in the colon. Further supporting this explanation is that various reducing reactions, such as nitro, azo, and arene reduction require an anaerobic environment (Hayes et al., 1996). If an environment with decreased oxygen enhances QR activity this may have contributed, along with the possible increased levels of quercetin, to the dramatic increase of QR activity in the colon, which is known to be an anaerobic environment (Roediger, 1980).

Genistein, however, did not result in significant changes in QR activity (Table 8). These results are in agreement with Breinholt et al. (1999), but are in contrast to two studies by Appelt and Reicks (1997 & 1999) showing that a similar range of genistein intake when consumed as soy protein isolate or soy flour did increase quinone reductase activity. This suggests that it is not isolated genistein that increases the activity of colon QR, but other components of soy, possibly other isoflavones or amino acids, that elicit an increase in QR colon activity.

Quercetin did not have a significant impact on lung QR or GST activity. Since the tissue concentration of quercetin, or its metabolites, were not measured in the tissues it is impossible to determine whether quercetin was absorbed into the tissue and in what form, or if whether it made it through "first pass effect" of the small intestine and liver in order to reach circulation. It is probable that a sufficient concentration of quercetin needed to induce activity was not absorbed into the lung tissue or it was not in a bioactive form.

Genistein did not alter lung GST, but after 28 day of feeding decreased QR. This may have occurred as a result of the activity of other enzymes that were not measured and may have been induced by genistein. By 28 days the concentration of genistein metabolites or competitive inhibition by other enzymes may have exhausted the available supply of NAD(P)H used by QR to carry out its 2-electron reduction (Oesch and Arand, 1999).

Neither flavonoid exerted much change in kidney QR activity, although all doses of genistein decreased QR activity after 14 day (Table 9). The results of the current study are in contrast to those of Appelt et al (1999 & 1997), who reported that soy protein

isolate and soy flour with genistein in a range that was lower than, or similar to my dietary concentrations, increased QR activity. As in the colon this suggests that more than aglycone genistein is needed to increase kidney QR. It is unclear by what mechanism aglycone genistein, or its metabolites, caused the decrease in QR activity seen in the lung.

The lack of QR induction in quercetin treated animals may be because the metabolites of quercetin that reached the kidneys were unable to induce QR activity. For example, quercetin-glucuronide, has been identified as the major plasma metabolite in rats and humans (Sesink et al., 2001).   Another explanation is that quercetin may not have been absorbed systemically and therefore was not excreted in the urine via kidneys, but rather it was conjugated in the small intestine or liver and because of its molecular weight was released into the bile and excreted via the colon. Glucuronide conjugates with a molecular weight less than 250, are commonly excreted in the urine by the kidneys (Klaassen et al., 1986). A glutathione or glucuronide conjugate, with a molecular weight greater than 350 is excreted in bile (Klaassen et al., 1986). The molecular weight of quercetin is 338, which possibly makes it a candidate for either route of excretion.

The structural differences between quercetin and genistein may explain their contrasting effects on QR activity. It has been reported that inducers of QR are planar aromatics and antioxidant polyphenols (Jaiswal, 1994). Both genistein and quercetin fall into this classification (Breinholt et al., 1999; Mitchell, 1998). However, it has been reported that the phenol must be in the para (1,4) or ortho (1,2) positions for QR to carry out its 2-electron reduction to form a stable hydroquinone (Talalay et al., 1988). On the B ring of the aglycone quercetin the hydoxyl groups are attached in an ortho orientation (Figure 2), and its two more common metabolites, isorhamnetin and tamarixten also have

substituents in the ortho positions on the B ring (Manach et al., 1996). Conversely, the B

ring phenol within the aglycone genistein is in the meta (1,3) position, although its

proposed colonic bacterial derived metabolite, 4-ethylphenol, is in a para orientation. If

structure is a determinant of QR induction then quercetin and its two metabolites

structurally appear to have a greater capacity to induce QR activity compared to

genistein.

An explanation as to why genistein significantly decreased QR activity in the

liver, lung, and kidneys could be because it may have induced the activities of enzymes

that also use NAD(P)H as their electron donors. For example competitive inhibition of

QR has been known to occur upon the introduction of dicumarol, which is a known to

inhibit QR activity in respect to NAD(P)H availability (Cadenas, 1995).

Cytochrome P450 1A1

The basal level of CYP1A1 is normally low unless induced by dietary or

environmental inducers (Guengerich, 1993). The results of this study demonstrated that

the addition of the flavonoids either decreased CYP1A1 activity or had no effect in the

liver, colon, and lung tissues.

There were no significant changes in liver CYP1A1 activity after exposure to

genistein or quercetin in this study, similar to the results of other studies (Breinholt et al.,

1999; Brouard et al., 1988; Canivenc-Lavier, 1996; Siess, 1989). However, some groups

have fed rat's soy protein isolate and have shown reduced activity of carcinogen-induced

CYP1A1 activity in rats (Ronis et al., 2001; Rowlands et al., 2001). These studies used

higher doses of genistein (~ 5.5 mg/d) than used in the current study (Ronis et al., 2001;

Rowlands et al., 2001). The study by Mirsalis et al (1993) found that soya bean flour

with doses of genistein ranging from 0.01 mg/d to 4 mg/d, decreased hepatic CYP1A1

activity after a 30d feeding experiment. This suggests that soy components other than

genistein may be responsible for the effects on CYP1A1 activity.

To our knowledge, the effect of aglycone quercetin and genistein on colon mucosa

CYP1A1 activity has not been previously measured *in vivo*. In this study, quercetin

decreased CYP1A1 activity slightly in a dose response manner. The lack of change in

lung CYP1A1 activity could be a result of low circulating or tissue concentrations of the

bioactive flavonoids.

This study has demonstrated the ability of quercetin and genistein to act as mono-

functional inducers at several doses, in different tissues, and at varying time points. The

interaction of the flavonoids with the ARE rather than the XRE gene region provides an

explanation of the results observed in this study. Inducing only the ARE has been found

to stimulate the transcription of certain PII enzymes, but not CYP1A1 (Jaiswal, 2000).

The molecular structure of quercetin and genistein may help explain why neither

quercetin or genistein significantly increased CYP1A1 activity. Since we did not

measure gene expression it is impossible to conclude whether the effect of the flavonoids

was caused at the level of the Ah receptor mediated gene transcription or by direct

enzyme inhibition. Direct enzyme inhibition could also have been caused by the

alteration in expression of other enzymes affected by the presence of the flavonoids or

their metabolites.

The ability of the flavonoids to inhibit CYP1A1 activity, and other

monofunctional oxidase enzymes, has been credited to some of the same structural

characteristics as those which allow the flavonoids to act as antioxidants (Cook and Samman, 1996; Rajinarayanan et al., 2001; Siess et al., 1995). The bioactive structural characteristics of quercetin and genistein include the double bond between C2-C3 (Siess et al., 1995) and the hydroxyl groups at the C5 and C7 positions (Rajinarayanan et al., 2001). The presence of many hydroxyl groups has generally been found to increase inhibition potency (Chae et al., 1991), and their planar conformation has a role in inhibiting activity (Siess et al., 1995). As mentioned previously, an important structural difference between quercetin and genistein is the ortho orientation of the hydroxyl groups on the B ring, which may have a role in altering enzyme activity. The ortho position of the hydroxyl groups on the quercetin compound was found to be a more potent inhibitor of CYP1A1 activity compared to morin, a flavonoid with the same structure as quercetin except for the meta orientation of the hydroxyl groups on the B ring (Tsyrlov et al., 1994). As seen with QR activity, quercetin had a greater impact of decreasing CYP1A1 activity in the colon than genistein, which could be due to quercetin's ortho position of hydroxyl groups, and its possible increased concentration in the colon (Manach et al., 1997). The increase in colon QR activity and decrease in colon CYP1A1 activity at all doses and time points parallel each other.

Genistein exposure did not alter CYP1A1 activity. The lack of change in CYP1A1 activity may have been caused by genistein binding to the ARE gene region or altering the Ah receptor. It has been suggested that genistein's ability to inhibit TCDD induced CYP1A1 activity was via the inhibition of tyrosine kinase activity, which in turn altered phosphorylation of heat shock protein 90 in the cytosolic Ah receptor complex resulting in impaired translocation of the Ah receptor into the nucleus (Gradin et al.,

1994). More recently Rowlands et al. (2001) discovered that Sprague-Dawley rats fed

soy protein isolate had greater Ah receptor and ARNT mRNA levels, but lower Ah

receptor and ARNT protein levels in hepatic and mammary tissues. Their results suggest

that soy protein isolate may inhibit the expression of Ah receptor and ARNT proteins

with the final outcome being reduced CYP1A1 expression (Rowlands et al., 2001). We

cannot draw any definite conclusion because gene expression of the ARE, XRE, or Ah

receptors were not measured in this study.

In summary, this study demonstrated the ability of quercetin and genistein to act

as mono-functional inducers. There was evidence of dose response relationships between

flavonoid concentrations and changes in enzyme activity in the colon of rats treated with

quercetin. This ability to mono-functionally induce PII enzymes could provide protection

against carcinogenesis, particularly in the colon.

TABLE 2

Composition of Experimental Diet [1,2]

INGREDIENT	AMOUNT (g/kg diet)
Casein	200
Cornstarch	529.5
Sucrose	100
Soybean Oil	70
Cellulose	50
AIN-93G Vitamin Mix[3]	10
AIN-93G Mineral Mix †	35
Quercetin	*
Genistein	*
L-Cystine	3
Choline Bitartrate	2.5

[1] AIN-93 diet *(Reeves et al. (1993)*

[2] Diet components, excluding flavonoids, were purchased from Harlan Teklad Diets (Madison, WI)

*Amount of flavonoid added; Quercetin: 3 g/kg, 6 g/kg, 9 g/kg
Genistein: 0.008 g/kg, 0.0016 g/kg, 0.012 g/kg

TABLE 3

Food intake of rats were fed low, medium, or high doses of quercetin or genistein for four weeks[1, 2, 3]

Dietary Treatment	Food Intake 1 (g/d)	Food Intake 2 (g/d)	Food Intake 3 (g/d)	Food Intake 4 (g/d)
Control	14.6 ± 0.2^a	17.0 ± 0.4	18.7 ± 0.5	19.5 ± 0.5
QC1	12.5 ± 0.1^b	17.4 ± 0.4	18.3 ± 0.4	19.0 ± 0.6
QC2	12.4 ± 0.1^b	17.7 ± 0.3	18.6 ± 0.2	18.5 ± 0.7
QC3	12.6 ± 0.1^b	18.2 ± 0.7	19.5 ± 0.5	19.3 ± 0.5
ANOVA	0.0001	NS	NS	NS
Control	14.6 ± 0.1^a	17.0 ± 0.4^a	18.7 ± 0.6	19.5 ± 0.6
GS1	12.5 ± 0.1^b	18.1 ± 0.4^b	18.6 ± 0.5	19.1 ± 0.6
GS2	12.6 ± 0.1^b	17.8 ± 0.2^a	19.2 ± 0.5	19.6 ± 0.6
GS3	12.5 ± 0.1^b	18.4 ± 0.3^b	18.7 ± 0.4	19.4 ± 0.2
ANOVA	0.0001	0.04	NS	NS

[1] Means $\pm$ SEM (n=7-8/ group), for each flavonoid within a given column with different superscript letters differ significantly (p<0.05).

[2] Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9%quercetin, GS1=0.0008% genistein, GS2=0.0012% genistein, GS3=0.002% genistein

[3] Food intake was measured at four different time points for three consecutive days.

TABLE 4

Initial and final body weights of rats. Rats were fed low, medium, or high doses of quercetin or genistein [1,2,3]

	Dietary Treatment	Initial Body Weight (g)	Final Body Weight (g)
14 Days			
	Control	72±4	171±4
	QC1	73±4	170±3
	QC2	73±4	178±5
	QC3	73±3	168±4
	Control	72±3	171±4
	GS1	71±3	164±4
	GS2	73±2	168±3
	GS3	71±2	167±3
28 Days			
	Control	74±3	251±11
	QC1	72±2	256±7
	QC2	73±2	252 ±8
	QC3	75±2	265±7
	Control	74±3	251±11
	GS1	73±2	261±4
	GS2	72±2	262±5
	GS3	73±2	255±4

[1] Means ± SEM (n=7-8/ group). There were no significant effects of diet on body weight.
[2] Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012% genistein, GS3=0.002% genistein
[3] Body weight was measured weekly.

TABLE 5

Liver to body weight ratio of rats. Rats were fed low, medium, or high doses of quercetin or genistein [1,2]

Dietary Treatment	14 Days (g)	28 Days (g)
Control	3.51 ± 0.102^b	2.85 ± 0.153
QC1	4.21 ± 0.312^a	2.94 ± 0.109
QC2	3.39 ± 0.072^b	2.97 ± 0.116
QC3	3.83 ± 0.170^b	3.14 ± 0.199
ANOVA	p= 0.02	p= NS
Control	3.51 ± 0.102	2.84 ± 0.153
GS1	3.76 ± 0.245	3.28 ± 0.292
GS2	3.64 ± 0.305	3.27 ± 0.226
GS3	3.78 ± 0.188	3.21 ± 0.229
ANOVA	p= NS	p= NS

[1] Means $\pm$ SEM (n=7-8/group), within a given column, with different superscript letters differ significantly ($p<0.05$).

[2] Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012% genistein, GS3=0.002% genistein

TABLE 6

Mean Glutathione-S-Transferase activity of liver and colon mucosa tissues. Rats were treated with low, medium, or high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
LIVER	U/mg Protein	
Control	0.093 ± 0.017^{b}	0.126 ± 0.012^{ab}
QC1	0.113 ± 0.010^{b}	0.081 ± 0.012^{c}
QC2	0.120 ± 0.008^{b}	0.143 ± 0.017^{a}
QC3	0.160 ± 0.013^{a}	0.092 ± 0.009^{bc}
ANOVA	p= 0.006	p= 0.005
Control	0.088 ± 0.018^{b}	0.125 ± 0.013^{bc}
GS1	0.149 ± 0.014^{a}	0.164 ± 0.013^{ab}
GS2	0.139 ± 0.018^{a}	0.199 ± 0.025^{a}
GS3	$0.084+0.016^{b}$	$0.099+0.017^{c}$
ANOVA	p= 0.015	p= 0.003
COLON MUCOSA		
Control	0.148 ± 0.025^{b}	0.218 ± 0.035
QC1	0.085 ± 0.033^{b}	0.255 ± 0.043
QC2	0.258 ± 0.045^{a}	0.311 ± 0.065
QC3	0.281 ± 0.031^{a}	0.218 ± 0.048
ANOVA	p= 0.001	NS
Control	0.148 ± 0.025	0.218 ± 0.035^{ab}
GS1	0.233 ± 0.038	0.189 ± 0.019^{ab}
GS2	0.231 ± 0.039	0.134 ± 0.037^{b}
GS3	0.265 ± 0.043	0.300 ± 0.066^{a}
ANOVA	NS	p= 0.071

[1]Means $\pm$ SEM (n=7-8/ group). For each flavonoid within a column with different superscript letters differ significantly (p<0.05); there was a time effect on enzyme activity, from 14 days to 28 days, in the liver, for both QC and GS (p=0.01), and a trend in the colon (p= 0.06), of QC treated rats.
[2]Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012%, genistein, GS3=0.002% genistein
[3] One unit of GST activity is defined as nmole conjugate formed per minute

TABLE 7

Mean Glutathione-S-Transferase activity of lung and kidney tissues. Rats were treated with low, medium, or high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
	U/mg Protein	
LUNG		
Control	0.012 ± 0.002^{ab}	0.011 ± 0.001
QC1	0.009 ± 0.001^{b}	0.011 ± 0.001
QC2	0.009 ± 0.001^{b}	0.012 ± 0.001
QC3	0.013 ± 0.001^{a}	0.012 ± 0.002
ANOVA	p= 0.027	NS
Control	0.012 ± 0.002^{ab}	0.011 ± 0.001
GS1	0.011 ± 0.001^{ab}	0.011 ± 0.001
GS2	0.009 ± 0.001^{b}	0.010 ± 0.001
GS3	0.014 ± 0.002^{a}	0.010 ± 0.001
ANOVA	p= 0.121	NS
KIDNEY		
Control	0.016 ± 0.001^{ab}	0.020 ± 0.004
QC1	0.016 ± 0.002^{ab}	0.017 ± 0.001
QC2	0.018 ± 0.001^{a}	0.022 ± 0.002
QC3	0.013 ± 0.002^{b}	0.022 ± 0.003
ANOVA	p= 0.150	NS
Control	0.020 ± 0.002	0.013 ± 0.001^{b}
GS1	0.019 ± 0.001	0.022 ± 0.002^{a}
GS2	0.019 ± 0.001	0.015 ± 0.002^{b}
GS3	0.019 ± 0.001	0.016 ± 0.002^{b}
ANOVA	NS	p= 0.009

[1]Means (n=7-8/ group). For each flavonoid within a column with different superscript letters differ significantly (p<0.05); there was a time effect on enzyme activity, from 14 days to 28 days, in the kidney, QC (p=0.004) and GS (p=0.02), of treated rats.
[2]Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012% genistein, GS3=0.002% genistein
[3] One unit of GST activity is defined as nmole conjugate formed per minute

TABLE 8

Mean Quinone Reductase activity of liver and colon mucosa tissues. Rats were treated with low, medium, or high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
LIVER	U/mg Protein	
Control	0.17 ± 0.02^{b}	0.07 ± 0.01^{b}
QC1	0.16 ± 0.03^{b}	0.09 ± 0.01^{b}
QC2	0.14 ± 0.01^{b}	0.09 ± 0.01^{b}
QC3	0.31 ± 0.05^{a}	0.19 ± 0.02^{a}
ANOVA	p= 0.001	p= 0.0001
Control	0.17 ± 0.02^{a}	0.07 ± 0.01
GS1	0.11 ± 0.01^{b}	0.08 ± 0.02
GS2	0.09 ± 0.02^{b}	0.05 ± 0.01
GS3	0.01 ± 0.01^{b}	0.06 ± 0.01
ANOVA	p= 0.007	NS
COLON MUCOSA		
Control	3.25 ± 0.39^{b}	2.95 ± 0.79^{b}
QC1	$6.66+0.78^{c}$	4.86 ± 0.51^{b}
QC2	9.18 ± 1.71^{c}	8.27 ± 1.01^{a}
QC3	15.9 ± 1.22^{a}	9.29 ± 1.25^{a}
ANOVA	p= 0.0001	p= 0.0002
Control	3.25 ± 0.38^{ab}	2.95 ± 0.79
GS1	3.26 ± 0.37^{ab}	2.05 ± 0.27
GS2	4.38 ± 1.21^{a}	2.27 ± 0.39
GS3	1.93 ± 0.44^{b}	2.01 ± 0.51
ANOVA	p= 0.130	NS

[1] Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin, QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012%, genistein, GS3=0.002% genistein

[2] Means $\pm$ SEM (n=7-8/ group). For each flavonoid within a column with different superscript letters differ significantly (p<0.05); there was a time effect on enzyme activity, from 14 days to 28 days in the liver, QC and GS (p= 0.0001) and colon mucosa, QC (p=0.002) and GS (p=0.05),of treated rats.

[3] One unit of QR activity is defined as nmol 2,6-Dichlorophenolindophenol reduced/min per mg protein

TABLE 9

Mean Quinone Reductase activity of lung and
kidney tissues. Rats were treated with low, medium, or
high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
	U/mg Protein	
LUNG		
Control	1.34 ± 0.07	1.03 ± 0.08
QC1	1.33 ± 0.07	0.94 ± 0.08
QC2	1.20 ± 0.08	1.0 ± 0.06
QC3	1.42 ± 0.21	0.87 ± 0.04
ANOVA	NS	NS
Control	1.38 ± 0.07^a	1.0 ± 0.08^a
GS1	1.15 ± 0.06^{ab}	0.72 ± 0.05^b
GS2	0.847 ± 0.12^c	0.69 ± 0.06^b
GS3	1.12 ± 0.06^b	0.77 ± 0.10^b
ANOVA	p= 0.001	p= 0.015
KIDNEY		
Control	0.11 ± 0.02^a	0.07 ± 0.01
QC1	0.12 ± 0.01^a	0.24 ± 0.12
QC2	0.12 ± 0.03^a	0.09 ± 0.01
QC3	0.05 ± 0.01^b	0.11 ± 0.01
ANOVA	p= 0.012	NS
Control	0.16 ± 0.03^a	0.083 ± 0.01
GS1	$0.09+0.01^b$	0.074 ± 0.00
GS2	$0.10+0.01^b$	0.071 ± 0.00
GS3	0.09 ± 0.01^b	0.085 ± 0.01
ANOVA	p= 0.013	NS

[1]Means (n=7-8/group). For each flavonoid, within a column
with different superscript letters differ significantly
(p<0.05); there was a time effect on enzyme activity, from
14 days to 28 days in the kidney, QC (p=0.004) and GS (p= 0.016),
of treated rats.
[2]Abbreviations: QC1=0.3% quercetin, QC2=0.6% quercetin,
QC3=0.9% quercetin, GS1=0.0008% genistein, GS2=0.0012%
genistein, GS3=0.002% genistein
[3] One unit of QR activity is defined as nmol 2,6-Dichlorophenolindophenol
reduced/min per mg protein

TABLE 10

Mean Cytochrome P450 1A1 activity of liver microsomes. Rat were treated with low, medium or high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
	pmol/(min/mg protein)	
LIVER		
Control	12.3 ± 1.4^{ab}	16.1 ± 2.7^{ab}
QC1	$9.3 \pm 0.9^{b*}$	18.5 ± 1.1^{a}
QC2	12.2 ± 1.4^{ab}	13.4 ± 2.4^{ab}
QC3	16.4 ± 2.8^{a}	11.8 ± 1.7^{b}
ANOVA	p= 0.06	p= 0.14
Control	12.3 ± 1.4	16.1 ± 2.7
GS1	9.8 ± 1.2	12.7 ± 1.9
GS2	10.0 ± 1.0	13.0 ± 2.0
GS3	10.3 ± 1.5	11.2 ± 1.0
ANOVA	NS	NS

[1] Means $\pm$ SEM (n=7-8/ group). For each flavonoid within a column with different superscript letters differ significantly (p<0.05); there was a time effect on enzyme activity, from 14 days to 28 days, for QC treated rats (p = 0.03).

[2] Abreviations: QC1= 0.3% quercetin, QC2 = 0.6% quercetin, QC3= 0.9% quercetin, GS1 = 0.0008% genistein, GS2 = 0.0012% genistein, GS3= 0.002% genistein

[3] As fluorescence was measured there was an increase in absorbance throughout the time of the reaction in all of the treatment groups. The rate of change in enzyme activity may be greater or less than control.

[4] One unit of CYP1A1 activity is defined as pmol resorufin formed/mg microsomal protein per min

TABLE 11

Mean Cytochrome P450 1A1 activity decreases in the colon mucosa and lung microsomes after treatment with low, medium or high doses of quercetin or genistein[1,2,3]

Dietary Treatment	14 Days	28 Days
	pmol/(min×mg protein)	
COLON MUCOSA		
Control	6.4 ± 0.9^c	6.3 ± 1.2^b
QC1	10.2 ± 1.9^{bc}	13.1 ± 1.6^b
QC2	13.9 ± 1.9^{ab}	12.9 ± 1.4^b
QC3	18.0 ± 3.1^a	30.4 ± 5.5^a
ANOVA	p=0.003	p=0.0001
Control	6.4 ± 0.9	6.3 ± 1.2
GS1	7.3 ± 1.4	9.4 ± 1.3
GS2	6.4 ± 1.3	12.0 ± 5.6
GS3	6.7 ± 2.0	12.3 ± 5.3
ANOVA	NS	NS
LUNG		
Control	2.3 ± 0.2^a	2.3 ± 0.6
QC1	2.6 ± 0.5^a	1.9 ± 0.3
QC2	2.1 ± 0.2^{ab}	1.9 ± 0.1
QC3	1.3 ± 0.2^b	2.2 ± 0.2
ANOVA	p= 0.03	NS
Control	2.3 ± 0.2	2.3 ± 0.6
GS1	2.0 ± 0.3	2.1 ± 0.2
GS2	2.0 ± 0.4	2.4 ± 0.3
GS3	1.6 ± 0.1	2.1 ± 0.2
ANOVA	NS	NS

[1] Means $\pm$ SEM (n=7-8/ group). For each flavonoid, within a column with different superscript letters differ significantly (p<0.05); there was a time effect on enzyme activity, from 14 days to 28 days in the colon (p= 0.05) of QC treated rats.

[2] As fluoresence was measured there was a decrease in absorbance during the time of the reaction in all of the of the treatment groups, within both the colon and lung tissues. A higher number indicates a greater decrease in enzyme activity.

TABLE 12. Significant Changes in Enzyme Activity of Animals Treated with Low, Medium, or High Doses of Quercetin or Genistein[1]

Organ	Enzyme	Activity at 14d Quercetin	Activity at 14 Genistein	Activity at 28 Quercetin	Activity at 28d Genistein
LIVER	GST	↑QC3	↑GS1, ↑GS2		
	QR	↑QC3	↓GS1 ↓GS2 ↓GS3	↑QC3	↓GS1 ↓GS2 ↓GS3
	CYP1A1				
COLON	GST	↑QC2 ↑QC3			
	QR	↑QC1 ↑QC2 ↑QC3		↑QC2, ↑QC3	
	CYP1A1	↓QC2 ↓QC3		↓QC3	
KIDNEY	GST				
	QR		↓GS1 ↓GS2, ↓GS3		
LUNG					
	GST				
	QR				↓GS1 ↓GS2 ↓GS3
	CYP1A1				

[1] (↑) Signifies an increase in enzyme activity and (↓) signifies a decrease in enzyme activity

Figure 9

14d QCT Mucosa QR Activity
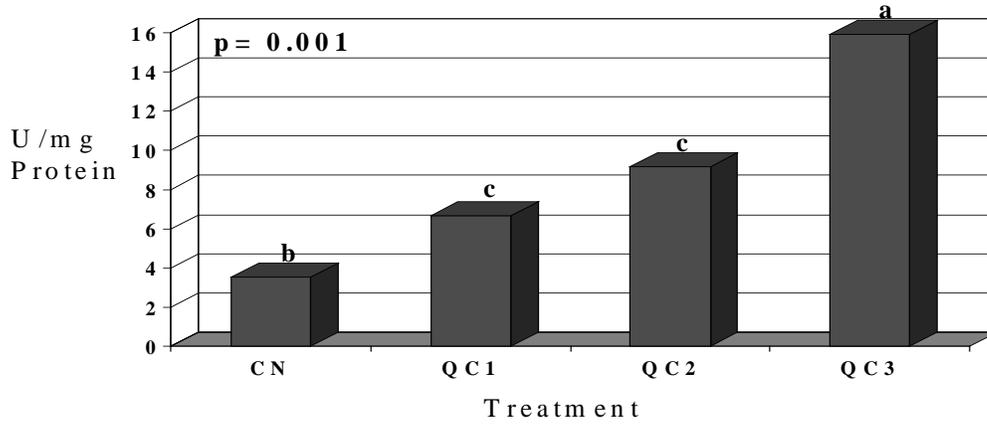
14 day quinone reductase activity within the colon mucosa of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 10

28d QCT Mucosa QR Activity
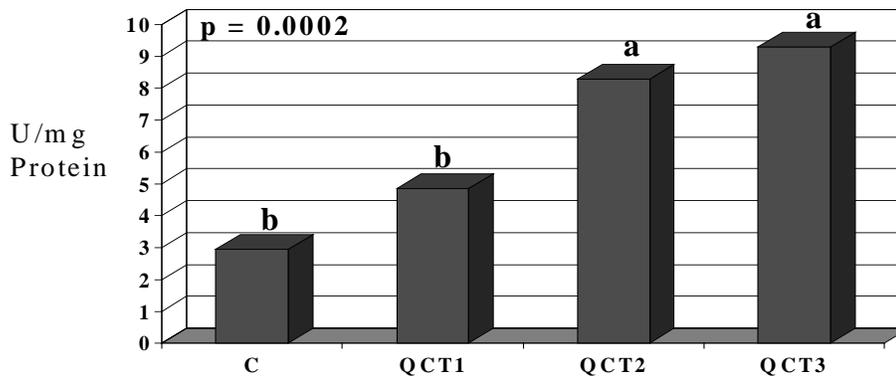
28 day quinone reductase activity within the colon mucosa of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 11

14d QC Liver QR Activity
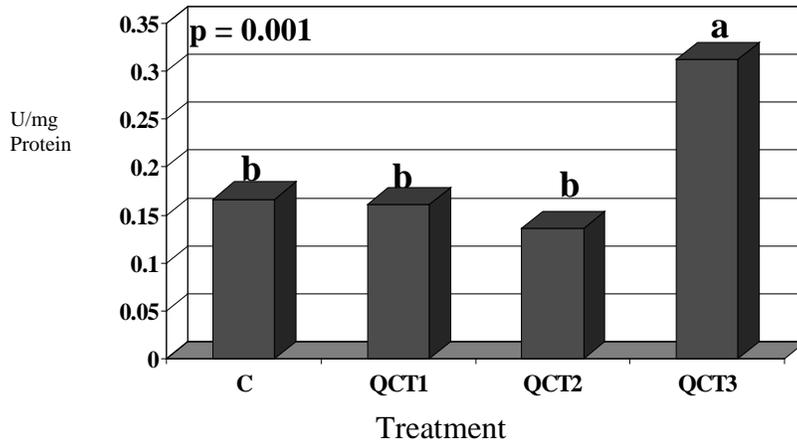
14 day quinone reductase activity within the liver of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 12

28d QC Liver QR Activity
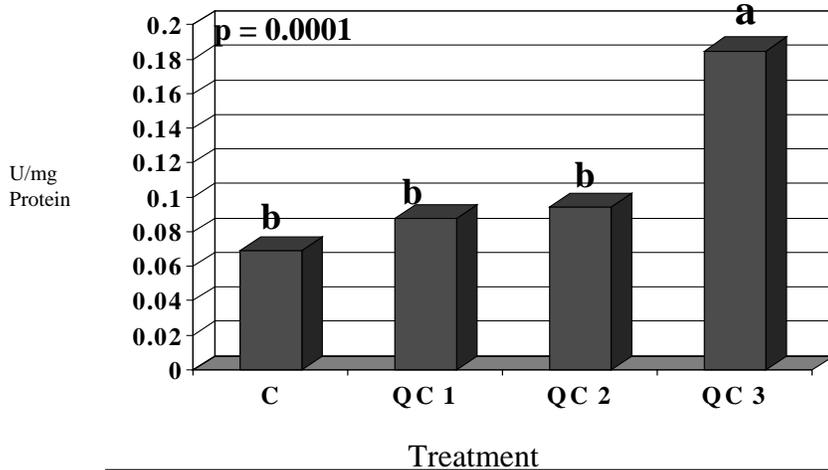
28 day quinone reductase activity within the liver of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 13

14d GS Liver QR Activity
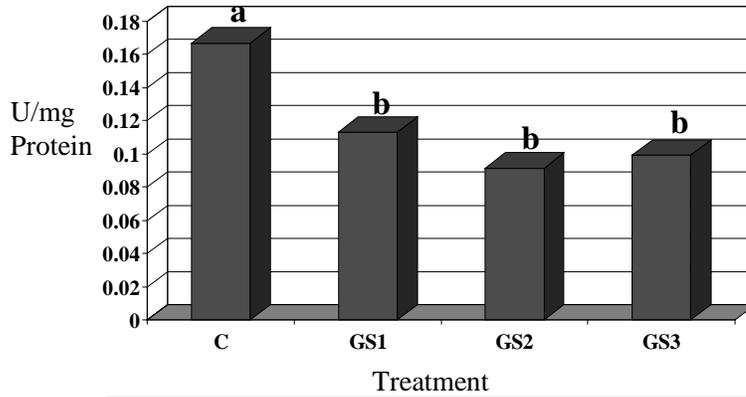
14 day week Quinone Reductase activity within the liver of rats treated with low (0.0008%), medium (0.0012%), or high (0.002%) doses of genistein. Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 14

28d GS Lung QR Activity
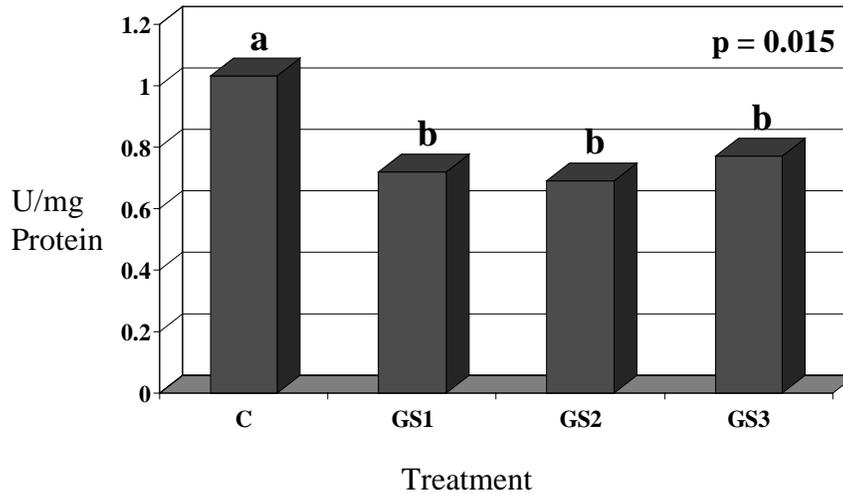
14 day week Quinone Reductase activity within the liver of rats treated with low (0.0008%), medium (0.0012%), or high (0.002%) doses of genistein. Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 15

14d GS Kidney QR Activity
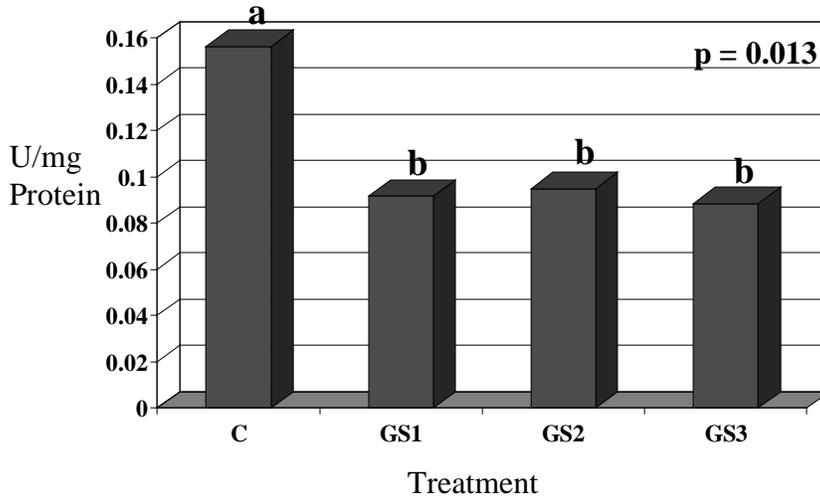
14 day week Quinone Reductase activity within the kidney of rats treated with low (0.0008%), medium (0.0012%), or high (0.002%) doses of genistein.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 16   ## 14d QC Liver GST Activity
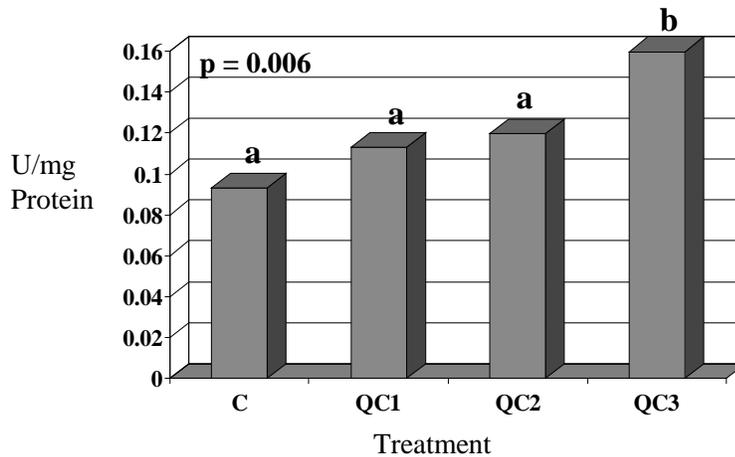
14 day glutathioe-s-transferase activity within the liver of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 17

14d GS Liver GST Activity
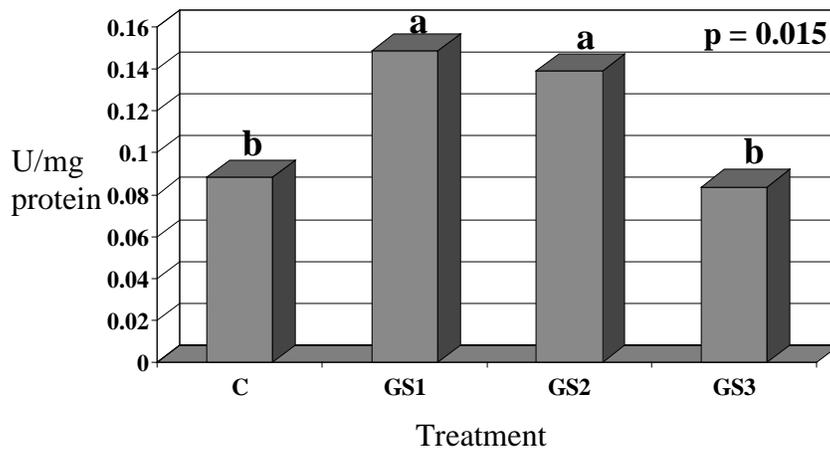
14 day week glutathione-s-transferase activity within the liver of rats treated with low (0.0008%), medium (0.0012%), or high (0.002%) doses of genistein. Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 18
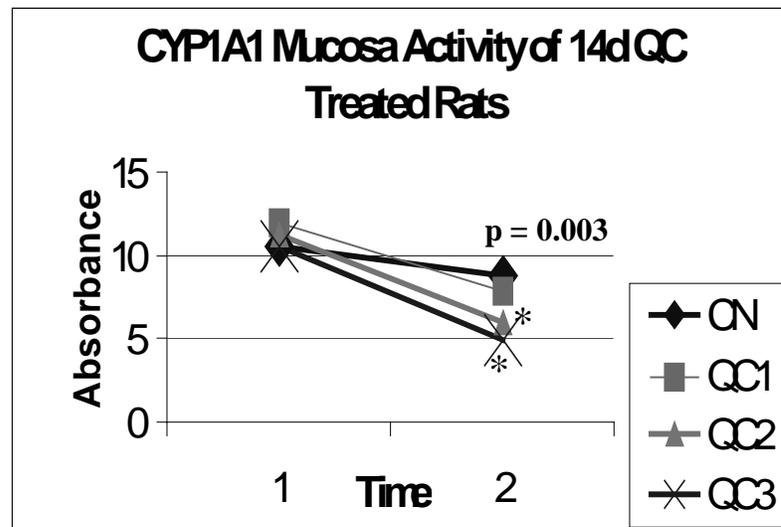
14 day cytochrome P450 activity within the mucosa of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

Figure 19

CYP1A1 Mucosa Activity of 28d QC Treated Rats
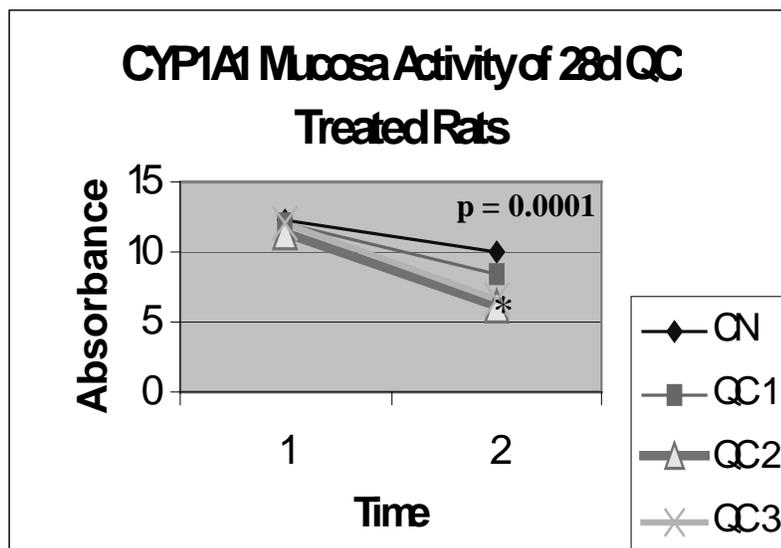
28 day cytochrome P450 1A1 activity within the mucosa of rats treated with low (0.3%), medium (0.6%), or high (0.9%) doses of quercetin.
Means (n=7-8group) within a column with different superscript letters differ significantly (p<0.05)

CHAPTER 4

SUMMARY

<u>Major Findings</u>

QR and GST enzyme activities were not always increased in the lung, liver, colon, and kidneys as a result of dietary exposure to either quercetin or genistein. Induction varied with compound, dose, tissue, and length of feeding. Both flavonoids were found to be mono-functional inducers in at least one of the tissues measured. Quercetin mono-functionally induced GST and QR activity in the liver and colon. Genistein mono-functionally induced GST activity in the liver. Enzyme activity was not maintained over the period of time from 14 to 28 day. There was evidence of a dose response increase in QR activity and somewhat of a dose response decrease in CYP1A1 activity within the colon of quercetin treated animals at 14 day, and dose response trends at 28 day. The tremendous difference between quercetin and genistein's effect on colon QR and CYP1A1 activity could be due to certain structural attributes of quercetin. The ortho orientation of the hydroxyl groups on the B ring of the polyphenol has been found to mono-functionally induce QR activity (Talalay et al., 1995). The lack or decrease of PII induction by genistein treatment could have been caused directly by its structure not inducing the enzyme or indirectly by altering other enzyme systems or the availability of cofactors needed to form the conjugating components needed by the enzymes. Another reason for the lack of PII enzyme induction in the lung and kidneys, by quercetin and genistein, may be due to inadequate circulation of the bioactive aglycone flavonoid, or bioactive metabolite.

Overall, this study demonstrated the ability of quercetin and genistein to function as mono-functional inducers of PII enzymes, which may be anti-carcinogenic by potentially reducing a tissue's exposure to reactive carcinogens (Klaassen et al., 1986).

Implications

In the liver, lung, and kidneys there were no evident patterns of mono-functional enzyme induction. The colon demonstrated more promising results with dose response changes in the activities of QR and CYP1A1. It is often difficult to establish patterns of enzyme activity due to the many factors affecting their activity (Klaassen et al., 1986). Adding to the variability of enzyme activity is the absorption and metabolism of the flavonoids. The tissue concentration of the parent flavonoids, or their metabolites needed to mono-functionally induce PII enzymes has not been established. At this point research has focused on studying the form of flavonoid, dose, length of feeding, and organ that can significantly alter enzyme activity. Currently, there are no established doses of flavonoids known to consistently induce, decrease, or inhibit the activity of an enzyme.

The animals in this study theoretically consumed either 60, 120, or 180 mg/d of quercetin. These doses cannot be reached in the human diet even for an individual who consumes a diet rich in fruits and vegetables. The lowest dose of quercetin is relative to a reference human of 70 kg consuming 19 g/d of quercetin. Currently, the higher end of human quercetin intake is estimated at 23 mg/d (Hertog et al., 1993). The dose of 19 g/d of quercetin is typically not even be reached with the use of supplements, with usual daily doses reaching 2 g of quercetin. However, the levels of quercetin used were within the range of a study that found quercetin supplementation at 0.45% to be protective against

the development of AOM induced abberant crypt foci (Warren et al., 2003). Furthermore, the doses in this study were well below the dose used by Wargovich et al. (1985) who found that 4% quercetin supplementation could reduce benzo{a}pyrene induce colonic nuclear damage. The doses used in this study were actually very low compared to what has been administered in the past to test quercetin's anti-carcinogenic mechanisms. This study did demonstrate that the low dose of quercetin supplementation was capable of inducing colon QR and decreasing CYP1A1 activity by a 2-fold difference from control, which could be important in the realm of discovering how quercetin exerts protection against carcinogen induced damage. It is our understanding that this is the first *in vivo* study to test aglycone quercetin's effect on colon QR and CYP1A1 activity without the addition of a carcinogen.

The low, medium, and high doses of genistein used in this study equated to the daily intake of 0.16, 0.2, or 0.4 mg/d of genistein. Using a 70 kg reference human the doses would correspond to a daily intake of 56, 70, or 126 mg of genistein. The medium and low doses are within the range of 20-80 mg/d, which is typically consumed in an Asian diet (Aldercreut et al., 1993). The low dose is somewhat higher than 24 mg/d, which is the typical amount of genistein consumed in the Western diet (Wang and Murphy, 1996). This study has shown that these doses of aglycone genistein did not significantly induce CYP1A1 in any of the tissues measured. Comparing these results to a study by Appelt and Reicks (1997) there appeared to be similar patterns of GST induction in the liver and colon, but contrasting effects of QR activity in the liver and kidney. In their follow up study (Appelt and Reicks, 1999) QR induction patterns were also different than the QR activity in the kidney and liver in the present study. These

results indicate that components, other than the aglycone genistein, found within soy

products could have an important influence on determining whether the activity of the

enzyme will change or have no effect. Currently, the interaction of various compounds,

including phytochemicals, within a whole food and their effect on systems *in vivo* is not

well understood. This study has shown that the aglycone flavonoids have anti-

carcinogenic potential by mono-functionally inducing PII enzymes *in vivo* that may

contribute to a fruit or vegetable's ability to elicit possible protection and prevention

against the development of chronic diseases. Humans are usually exposed to carcinogens

at smaller doses for either acute or chronic exposures. The enhanced activity of the

protective PII enzymes could provide defense against carcinogens, that humans are often

exposed to, by either reducing the formation of reactive compounds or quickly

eliminating them. Reducing the time a tissue is exposed to a harmful substance can be

protective against the development of chronic diseases, and amplified PII activity is key

in cancer protection.

Study Limitations

This study did not test quercetin or genistein's ability to mono-functionally induce

PII enzymes in a cancer model. The forms of quercetin and genistein tested were their

aglycone parent compounds, whereas humans consume the flavonoids within whole

foods attached to various sugar moieties. The doses of quercetin were well over what

could be obtained in the human diet or through supplemental use. Without measuring the

plasma and tissue concentrations of the flavonoids and their conjugates it is impossible to

know how much of the flavonoids were absorbed. It is not clearly understood at what

level the flavonoids were able to either increase or decrease enzyme activity. Without measuring gene or protein expression of the enzymes it is undetermined as to whether activity was altered at the level of transcription, substrate availability, or due to changes in other molecular pathways. Measuring the activity of only three enzymes limits the understanding of what is happening within the organism as a whole. For example while administering the large doses of quercetin we do not know if there were detrimental consequences to cells as a result of such high intakes, or if genistein caused changes due to their weak estrogenic properties. Choosing appropriate doses of flavonoids and time intervals to accommodate the inducible characteristics of the two PII enzymes when limited to 3 doses and two time points was challenging because the enzymes peak in response to different concentrations of flavonoids at different times. Therefore it is possible that with only two measurements and 3 doses, the activity of a particular enzyme may not have been induced due to negligible concentrations or activity not being measured at the time of its peak. There are many factors contributing to the high variability of enzyme activity and at this point any data that demonstrates an increase or decrease in enzyme activity is helpful in providing information on which enzyme is induced in which organ, and at what particular point in time.

Future Studies

It would be interesting to see if the flavonoids would have the same effect on enzyme activity if they were in whole foods, containing the same concentrations of the flavonoids used in this study. Since flavonoids exist as combinations in nature it would be worthwhile to combine the flavonoids in order to test their effect on enzyme activity in

different tissues. Continuing to research the mechanisms of metabolism and absorption of the flavonoids will provide the necessary information needed to determine the concentrations of metabolites that are absorbed into tissue. Deciphering the form and concentration of flavonoid needed to elicit a protective response in different organs is another area that requires more research. Also developing biomarkers capable of measuring the concentration of flavonoids would be useful when trying to associate decreased incidence of disease with flavonoid intake, such as in an intervention study.

Future research should continue to focus on time and dose responses of flavonoids in respect to their influence on activity of various PII enzymes because so little is known about the enzyme potential of each organ when exposed to different phytochemicals. It is important for humans to increase PII activity through fruit and vegetable consumption in order to combat the increased exposure to carcinogens associated with increased pollution.

REFERENCES

ACS (2002) Cancer prevention & early detection: Facts & Figures 2002. The American Cancer Society.

Agullo, G., Gamet, L., Besson, C., Demigne, C., & Remesy, C. (1994) Quercetin exerts a preverential cytotoxic effect on active dividing colon carcinoma HT29 and Caco-2 cells. Cancer Letters 87: 55-63.

Aldercreut, Z. H., Markkanen, H., & Wantabe (1993) Plasma concentrations of phytoestrogens in Japanese men. Lancet 342: 1109-1210.

Anlauer, W., Kolb, J., & Furst, P. (2000) Absorption and metabolism of genistin in the isolated rat small intestine. Federation of European Biochemical Societies 475: 127.

Anzenbacher, P., & Azenbacherova, E. (2001) Review: cytochromes P450 and metabolism of xenobiotics. Cellular and Molecular Life Sciences 58: 737-747.

Appelt, L. C., & Reicks, M. M. (1997) Soy feeding induces phase II enzymes in rat tissues. Nutrition and Cancer 28: 270-275.

Appelt, L. C., & Reicks, M. M. (1999) Soy induces phase II enzymes but does not inhibit dimethylbenz{a}anthracene-induced carcinogenesis in female rats. Journal of Nutrition 129: 1820-1826.

Arts, I. C., Hollman, P. C. H., Bueno De Mesquita, H. B., Feskens, E. J. M., & Kromhout, D. (2001) Dietary catechins and epithelial cancer incidence: the Zutphen elderly study. International Journal of Cancer 92: 298-302.

Auricchio, S., Rubino, A., Landolt, M., Smenza, G., & Prader, A. (1963) Lancet 2: 324-326.

Ayrton, A., & Morgan, P. (2001) Role of transport proteins in drug absorption, distribution and excretion. Xenobiotica 31: 469-497.

Aziz, A. A., Edwards, C. A., Lean, M. E., & Crozier, A. (1998) Absorption and excretion of conjugated flavonols, including uercetin-4'-O-beta-glucoside and isorhamnetin-4'-O-beta-glucoside by human volunteers after the consumption of onions. Free Radical Research 29: 257-269.

Barnes, S., Grubbs, C., Setchell, K. D. R., & Carlson, J. (1990) Soybeans inhibit mammary tumors in models of breast cancer. Progress in Clinical and Biological Research 347: 239-253.

Birt, D. F., Hendrich, S., & Wang, W. (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacology Therapeutics 90: 157-177.

Boersma, B. J. (2001) Soy isoflavonoids and cancer metabolism at the target site. Mutation Research 480-481: 121-127.

Bowey, E., Adlercreutz, I., & Rowland, C. (2002) Metabolism of isoflavones and lignans by the gut micro flora: a study in germ-free and human flora associated rats. Food and Chemical Toxicology 41: 631-636.

Bravo, L. (1998) Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56: 317-333.

Breinholt, V., Lauridsen, S. T., & Dragsted, L. O. (1999) Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rat. Xenobiotica 29: 1227-1240.

Brouard, C., Siess, M. H., Vernevaut, M. F., & Suschetet, M. (1988) Comparison of the effects of feeding quercetin or flavone on hepatic and intestinal drug-metabolizing enzymes of the rat. Food and Chemical Toxicology 26: 99-103.

Busby, M. G. (2002) Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. American Journal of Clinical Nutrition 75: 126-136.

Cadenas, E. (1995) Antioxidant and prooxidant functions of DT-Diaphorase in quinone metabolism. Biochemical Pharmacology 49: 127-140.

Cai, J., Huang, Z., & Lu, S. C. (1997) Differential regulation of y-glutamylcysteine synthetase heavy and light subunit gene expression. Biochemical Journal 326: 162-172.

Cai, Q., & Wei, H. (1996) Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. Nutrition and Cancer 25: 1-7.

Canivenc-Lavier, M.-C. (1996) Comparative effects of flavonoids and model inducers on drug-metabolizing enzymes in rat liver. Toxicology 114: 19-27.

Castillo, M. H., Perkins, E., Campbell, J. H., Doerr, R., Hassett, J. M., Kandaswami, C., & Middleton, E., Jr. (1989) The effects of the bioflavonoid quercetin on squamous cell carcinoma of head and neck origin. American Journal of Surgery 158: 351-355.

Catapano, A. L. (1997) Antioxidant effect of flavonoids. Angiology The Journal of Vascular Diseases 48: 39-44.

Chae, Y.-H., Marcus, C. B., Ho, D. K., Cassady, J. M., & Baird, W. M. (1991) Effects of synthetic and naturally occurring flavonoids on benzo[a]pyrene metabolism by hepatic microsomes prepared from rats treated with cytochrome P-450 inducers. Cancer Letters 60: 15-24.

Chang T. K. H., Waxman, C., & Waxman, D. J. (1998) Enzymatic analysis of cDNA-expressed human CYP1A1, CYP1A2, Nd CYP1B1 with 7-ethoxyresorufin as substrate. In I.R. Phillips, & E.A. Shepard, eds. Methods in Molecular CYP450 Protocols Humana Press, Rotowa.

Chen, J., Lin, H., & Hu, M. (2002) Metabolism of flavonoids via enteric recycling: Role of Intestinal Disposition. The Journal of Pharmacology and Experimental Therapeutics 304: 1228-1235.

Coldham, N. G., Howells, L. C., Santi, A., Montesissa, C., Langlais, C., King, L. C., Macpherson, D. D., & Sauer, M. J. (1999) Biotransformation of genistein in the rat: elucidation of metabolites structure by product ion mass fragmentology. Journal of Steroid Biochemistry 70: 169-184.

Coldham, N. G., & Sauer, M. J. (2000) Pharmcokinetics of {14C} Genistein in the Rat: Gender-Related Differences, Potential Mechanisms of Biological Action, and Implications for Human Health. Toxicology and Applied Pharmacology 164: 206-215.

Conquer, J. A., Maiani, G., Azzini, E., Raguzzini, A., & Holub, B. J. (1998) Supplementation with quercetin markedly increases plasma quercetin concentrations without effect on selected risk factors for heart disease in healthy subjects. Journal of Nutrition 128: 593-597.

Cook, N. C., & Samman, S. (1996) Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry 7: 66-76.

Daniels, L. B., Coyle, P. J., CHiao, Y., & Glew, R. H. (1981) Purification and characterization of a cytosolic specific broad specificity beta-glucoside from human liver. The Journal of Biological Chemistry 256: 13004-13013.

Day, J. A., Canada, J. F., Diaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., Plumb, G. W., Morgan, M. R. A., & Williamson, G. (2000) Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. Federation of European Biochemical Societies 468: 166-170.

De Stefani, E., Boffetta, P., Deneo-Pellegrini, H., Mendilaharsu, M., & Carzoglio, J. C. (1999) Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. Nutrition and Cancer 34: 100-110.

Deschner, E. E., Ruperto, J., Wong, G., & Newmark, H. L. (1991) Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 12: 1193-1196.

Deschner, E. E., Ruperto, J. F., Wong, G. Y., & Newmark, H. L. (1993) The effect of dietary quercetin and rutin on AOM-induced acute colonic epithelial abnormalities in mice fed a high-fat diet. Nutrtion and Cancer 20: 199-204.

Dinkova-Lostova, A. T., & Talalay, P. (2000) Persuasive evidence that quinone reductase type I (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. Free Radical Biology and Medicine 29: 231-240.

Fischer, J. G., & Fisher, H. L. (2000) Supplementation with the flavonoid quercetin alters the activity of some antioxidant enzymes. Journal of The American Dietetic Association, 100: (Suppl A11).

Fitzsimmons, J. T. R., Orson, N. V., & El-Aaaser, A. (1989) Effects of soybean and ascorbic acid on experimental carcinogenesis. Comparative Biochemistry and Physiology 93A: 285-290.

Fotsis, T., Pepper, M., Aldercreut, Z. H., Flerischmann, G., & Hase, T. A. (1993) Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proceedings of the National Academy of Sciences of the USA 90: 2690-2694.

Garcia-Closas, R., Gonzalez, C. A., Agudo, A., & Riboli, E. (1999) Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. Cancer Causes and Control 10: 71-75.

Garza, C., Murphy, S. P., Deckelbaum, R. J., Dwyer, J. D., & Grundy, S. M. (2000) Dietary Guidelines For Americans. United States Department of Agriculture United States Department of Health and Human Services, Washington DC.

Gee, J. M., Dupont, M. S., Rhodes, M. J. C., & Johnson, I. T. (1998) Quercetin glucosides interact with the intestinal glucose transport pathway. Free Radical Biology and Medicine 25: 19-25.

Goldbohm, R. A., Van Den Brandt, P. A., Hertog, M. G. L., Brants, H. A. M., & van Poppel, G. (1995) Flavonoid intake and risk of cancer: a prospective cohort study. American Journal of Epidemiology 41: 61s.

Gradin, K., Whitelaw, M. L., Toftgard, R., Poellinger, L., & Bergard, A. (1994) A tyrosine kinase-dependent pathway redulates ligand-dependent activation fo the dioxin receptor in human keratinocytes. Journal of Biological Chemistry 269: 23800-23807.

Guengerich, P. F. (1993) Cytochrome P450 enzymes. American Scientist 81: 440-448.

Guyton, A. C., & Hall, J. E. (2000) Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia, 1064 pages.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974) Glutathione-S-Transferase: the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 193: 265-274.

Haenszel, W., Kurihara, M., Segi, M., & Lee, R. K. C. (1972) Stomach Cancer among Japanese in Hawaii. Journal of the National Cancer Institute 49: 969-988.

Harborne, J. B. (1994) The flavonoids: advances in research since 1986. Chapman and Hall, New York, 621 pages.

Havsteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology 32: 1141-1148.

Hayes, W. S., Jenison, S. A., Yamada, T., Pastuszyn, A., & Glew, R. H. (1996) Primary structure of the cytosolic beta-glucosidase of guinea pig liver. The Biochemical Journal 319: 829-837.

Herrmann, K. (1976) Flavonols and flavones in food plants: A review. Journal of Food Technology 11: 433-448.

Herrmann, K. (1988) On the occurrence of flavonol and flavone glycosides in vegetables. Zeitschrift fur Lebensmittel-Untersuchungung-Forschung 186: 1-5.

Hertog, G. L. M., Hollman, P. C. H., Martijn, K. B., & Dromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutrition and Cancer 20: 21-29.

Hertog, G. L. M., Kromhout, D., Aravanis, C., Blackburn, H., & Buzina, R. (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine 155: 381-386.

Higashi, K., & Ogawara, H. (1992) Effects of isoflavone compounds on the activation of phopholipase C. Chemical and Pharmaceutical Bulletin 40: 157-160.

Hirayama, T. (1982) Relationship of soybean paste soup intake to gastric cancer risk. Nutrition and Cancer 3: 223-233.

Hollman, P. C. (1997) Bioavailability of flavonoids. Eur J Clin Nutr 51 Suppl 1: S66-69.

Hollman, P. C., & Katan, M. B. (1997) Absorption, metabolism and health effects of dietary flavonoids in man. Biomedicine Pharmacotherapy 51: 305-310.

Hollman, P. C., van Trijp, J. M., Mengelers, M. J., de Vries, J. H., & Katan, M. B. (1997) Bioavailability of the dietary antioxidant flavonol quercetin in man. Cancer Letters 114: 139-140.

Hollman, P. C., vd Gaag, M., Mengelers, M. J., van Trijp, J. M., de Vries, J. H., & Katan, M. B. (1996) Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Radical Biology and Medicine 21: 703-707.

Horn-Ross, P. L., John, E. M., & Lee, M. (2001) Phytoestrogen consumption and breast cancer risk in a multiethnic population: the bay area breast cancer study. American Journal of Epidemiology 154: 434-441.

Husain, S. R., Cillard, J., & Cillard, P. (1987) Hydrxyl radical scavenging activity of flavonoids. Phytochemistry 26: 2489-2491.

IARC (1988) International Agency for Research on Cancer, alcohol drinking. IARC Monogr Eval Carcinog Risks Hum 44: 194-220.

Ioannides, C. (1999) Effect of diet and nutrition on the expression of cytochromes P450. Xenobiotica 29: 109-154.

Jaiswal, A. K. (1994) Human NAD(P)H: quinone oxidoreductase 2. Gene structure, activity, and tissue-specific expression. Journal of Biological Chemistry 269: 14502-14508.

Jaiswal, A. K. (2000) Regulation of genes encoding NAD(P)H: Quinone oxidoreductases. Free Radical Biology and Medicine 29: 254-262.

Joannou, G. E., Kelly, G. E., Reeder, A. Y., Waring, M., & Nelson, C. A. (1995) Urinary profile study of dietary phytoestrogens: the identification and mode of metabolism of new isoflavonoids. Journal of Steroid Biochemistry Molecular Biology 54: 167-184.

King, R. A., Broadbent, J. L., & Head, R. J. (1996) Absorption and excretion of the soy isoflavone genistein in rats. Journal of Nutrition 126: 176-182.

Kiviranta, J., Huovinen, K., & Hiltunen, R. (1988) Variation of phenolic substances in onion. Acata Pharmaceutica Fennica 97: 67-72.

Klaassen, C. D., Amdudr, M. O., & Doull, J. (1986) Toxicology; The basic science of poisons. Macmillan publishing company, New York, p 88-126.

Knekt, P., Jarvinen, R., Seppanen, R., Heliovaara, M., & Teppo, L. (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. The American Journal of Epidemiology 146: 223-230.

Kore, A. M., Jeffery, E. H., & Wallig, M. A. (1993) Effects of 1-isothiocyanato-3-(methylsulfinyl)-propane on xenobiotic metabolizing enzymes in rats. Food and Chemical Toxicology 31: 721-729.

Kuhnau, J. (1976) The flavonoids; a class of semi-essential food components; their role in human nutrition. World Review of Nutrition and Dietetics 24: 117-191.

Kurzer, M. S., & Xu, X. (1997) Dietary Phytoestrogens. Annual Review of Nutrition 17: 353-381.

Lai, C., , & G, S. P. (1999) The role of interindividual variation in human carcinogenesis. Journal of Nutrition 129: 552S-555S.

Lee, H. P., Gourley, L., Duffy, S. W., Esteve, J., Lee, J., & Day, N. E. (1991) Dietary effects on breast-cancer risk in Singapore. The Lancet 337: 1197-1200.

Leese, H. J., & Semenza, G. (1973) On the identity between the small intestinal enzymes phlorizin hydrolase and glycosyleramidase. The Journal of Biological Chemistry 248: 8170-8173.

Liu, Y., & Hu, M. (2002) Absorption and metabolism of flavonoids in the caco-2 cell culture model and a perused rat intestinal model. Drug Metabolism and Disposition 30: 370-333.

Manach, C., Monrad, C., Texier, O., Favier, M. L., Agullo, G., Demigne, C., Regerat, F., & Remesy, C. (1995) Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. Journal of Nutrition 125: 1911-1922.

Manach, C., Texier, O., Regerat, F., Agullo, G., Demigne, C., & Remesy, C. (1996) Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin. Journal of Nutritional Biochemistry 7: 375-380.

Manach, C. M., C , Demigne, C., Texier, O., Regerat, F., & Remesy, C. (1997) Bioavailability of rutin and quercetin in rats. FEBS Letters 409: 12-16.

Marchand, L. L., Murphy, S. P., Hankin, J. H., Wilkens, L. R., & Kolonel, L. N. (2000) Intake of flavonoids and lung cancer. The Journal of the National Cancer Institute 92: 154-160.

Martin, J., Ronis, J., Rowlands, C., Hakkak, R., & Badger, T. M. (2001) Inducibility of Hepatic CYP1A enzymes by 3-methylcholanthrene and isosafrole differs in male rats fed diets containing casein, soy protin isolate or whey from conception to adulthood. Journal of Nutrition 131: 1180-1188.

Mauri, P. L., Lemoli, L., Gardana, C., Riso, P., Simonetti, P., & Porrini, P. G. (1999) Liquid chromatography/electrospray ionization mass spectrometric characterization of flavonol glycosides in tomato extracts and human plasma. Rapid Communications in Mass Spectrometry 13: 924-931.

McAnlis, G. T., McEneny, J., Pearce, J., & Young, I. S. (1999) Absorption and antioxidant effects of quercetin from onions, in man. European Journal of Clinical Nutrition 53: 92-96.

McMahon, L. G., Nakano, H., & Levey, M. D. (1997) Cytosolic pyridoxine-beta-D-glucoside hydrolase from porcine jejunal mucosa, purification, properties, and comparison with broad specificity beta-glucosidase. Journal of Biological Chemistry 272: 32025-32033.

Messina, M. J., Persky, V., Setchell, K. D. R., & Barnes, S. (1994) Soy Intake and Cancer Risk: A review of the In Vitro and In Vivo Data. Nutrition and Cancer 21: 113-131.

Mikulcik, E. M., & Fischer, J. G. (2001) Possible mechanism for protective effect of the flavonoid quercetin. Journal of The American Dietetic Association, 101: (Suppl): A35.

Mirsalis, J. C., Hamilton, C. M., Schindler, J. E., Green, C. E., & Dabbs, J. E. (1993) Effects of soya bean flakes and liquorice root extract on enzyme induction and toxicity in B6C3F1 mice. Food Chemistry and Toxicology 31: 343-350.

Mitchell, J. H. (1998) Antioxidant effecacy of phytoestrogens in chemical and biological model systems. Archives of Biochemistry and Biophysics 360: 142-148.

Mitchell, J. H. (2001) Phytoestrogens: involvement in breast and prostate cancer. In R.E.C. Wildman, ed. Handbook of nutraceuticals and functional foods, CRC Press, Boca Raton.

Monrad, C., Crespy, V., Manach, C., Bessen, C., Demigne, C., & Remesy, C. (1998) American Journal of Physiology 275: R212-R219.

Murphy, P. A. (1982) Phytoestrogen content of processed soybean products. Food Technology 43: 60-64.

Nagai, M., Hashimoto, T., Yanagawa, H., Yokoyama, H., & Minowa, M. (1982) Relationship of diet to the incidence of esophageal and stomach cancer in Japan. Nutrition and Cancer 3: 257-268.

Nebert, D. W., Roe, A. L., Dieter, M. Z., Solis, W. A., Yang, Y., & Dalton, T. P. (2000) Role of the Aromatic Hydrocarbon Receptor and {Ah} Gene Battery in the Oxidative Stress Response, Cell Cycle, and Apoptosis. Biochemical Pharmacology 59: 65-85.

Nomura, A., Henderson, B. E., & Lee, J. (1978) Breast cancer and diet among the Japanese in Hawaii. American Journal of Clinical Nutrition 31: 2020-2025.

Oesch, F., & Arand, M. (1999) Xenobiotic Metabolism. Academic Press, Sandiego.

O'Reilly, J. D., Mallet, A. I., McAnlis, G. T., Young, I. S., Halliwell, B., Sanders, T. A. B., & Wiseman, H. (2001) Consumption of flavonoids in onions and black tea: lack of effect on F2-isoprostanes and autoantibodies to oxidized LDL in healthy humans. American Journal of Clinical Nutrition 73: 1040-1044.

Paganga, G., & Rice-Evans, C. (1997) The identification of flavonoids as glycosides in human plasma. FEBS Letters 401: 78-82.

Pang, K. S. (1990) Kinetics of conjugation reactions in eliminating organs. In: G. J. Mulder (ed.) Conjugation Reactions in Drug Metabolism: An Integral Approach. Taylor & Francis Ltd, London, pp. 5-36.

Perera, F. P. (1997) Environment and cancer: Who are susceptible? Science 278: 1068-1073.

Pereira, M.A., Grubbs, C.J., Barnes, L.H., Li, H., Olson, G.R., Eto, I., Juliana, M., Whitaker, L.M., Kelloff, G.J., Steele, V.E., Lubet, R.A.(1996) Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz{a}anthracene-induced mammary cancer in rats. Carcinogenesis 17(6); 1305-1311

Peterson, T. G., Ji, G., Kirk, M., Coward, L., Falany, C. N., & Barnes, S. (1998) Metabolism of the isoflavones genistein and biochanin A in human breast cancer cell lines. American Journal of Clinical Nutrition 68: 1505s-1511s.

Pitot, H. C. (1986) Fundamentals of oncology. Marcel Dekker, New York.

Price, K. R., Bacon, J. R., & Rhodes, M. J. C. (1997) Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (Allium cepa). Journal of Agriculture and Food Chemistry 45: 938-942.

Rajinarayanan, R. V., Rowley, C. W., Hopkings, N. E., & Alworth, W. (2001) Regulation of pohenobarvital-Mediated induction of cyp102 (Cytochrome P450 BM-3) in Bacillus megaterium by phytochemicals from soy and green tea. Journal of Agriculture and Food Chemistry 49: 4930-4936.

Reis, L. A. G., Eisner, M. P., Kosary, C. L., Hanlcey, B. F., Miller, B. A., Clegg, L. X., & Edwards, B. K. (1999) SEER Cancer Statistics Review, 1973-1997. National Cancer Institute, Bethesda.

Rice-Evans, C., Miller, N. J., & Paganga, G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20: 933-956.

Rock, C. L., Lampe, J. W., & Patterson, R. E. (2000) Nutrition, genetics, and risks of cancer. Annuals Reviews of Public Health 21: 47-64.

Roediger, W. E. (1980) Anaerobic bacteria, the colon and colitis. The Australian and New Zealand Journal of Surgery 50: 73-75.

Ronis, M. J., Rowlands, C., Hakkak, R., & Badger, M. (2001) Inducibility of hepatic CYP1A enzymes by 3-Methylcholanthrene and isosafrole differs in male rats fed diets containing casein, soy portein isolate or whey from conception to adulthood. Journal of Nutrition 131: 1180-1188.

Ross, D. (2000) NAD(P)H: quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chemico-Biological Interactions 129: 77-97.

Rowlands, J. C., He, L., Hakkak, R., Ronis, M. J. J., & Badger, T. M. (2001) Soy and whey proteins downregulate DMBA-induced liver and mammary gland CYP1 expression in female rats. Journal of Nutrition 131: 3281-3287.

Scambia, G. (1994) Quercetin enhances transforming growth factor beta 1 secretion by human ovarian cancer cells. International Journal of Cancer 57: 211-215.

Schrenk, D. (1998) Impact of Dioxin-Type Induction of Drug Metabolising Enzymes in the Metabolism of Endo- and Xenobiotics. Biochemical Pharmacology 55: 1155-1162.

Sesink, A. L. A., O'Leary, K. A., & Hollman, P. C. H. (2001) Quercetin Glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4'-glucoside. Journal of Nutrition 131: 1938-1941.

Setchell, K. D., Brown, N. M., Desai, P., Zimmer-Nechemias, L., Wolfe, B. E., Brashear, W. T., Kirshner, A. S., Cassidy, A., & Heubi, J. E. (2001) Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. Journal of Nutrition 131: 1362s-1375s.

Setchell, K. D. R. (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. American Journal of Clinical Nutrition 68 suppl: 1333S-1346S.

Setchell, K. D. R. (2002) Evidence for lack of absorption of soy isoflavone glycosides inhumans, supporting the crucial role of intestinal metabolism for bioavailability. American Journal of Clinical Nutrition 16: 43-52.

Setchell, K. D. R. (2003) Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. Journal of Nutrition 133: 1027-1035.

Setchell, K. D. R., Faughnan, M. S., Avades, T., Zimmer-Nechemias, L., Brown, N. M., Wolfe, B. E., Brashear, W. T., Desai, P., Oldfield, M. F., Botting, N. P., & Cassidy, A. (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. American Journal of Clinical Nutrition 77: 411-419.

Severson, R. K., Nomura, A. M. Y., Grove, J. S., & Stemmermann, G. N. (1989) A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. Cancer Research 49: 1857-1860.

Sfakianos, J., Coward, L., Kirk, M., & Barnes, S. (1997) Intestinal Uptake and Biliary excretion of the isoflavone genistein in rats. Journal of Nutrition 127: 1260-1268.

Shertzer, H. G., Alvaro, P., Chang, C., Smith, P., Nebert, D. W., Setchell, K. D. R., & Dalton, T. P. (1999) Inhibition of CYP1A1 enzyme activity in mouse hepatoma cell culture by soybean isoflavones. Chemico-Biological Interactions 123: 31-49.

Siess, M.-H. (1989) Induction of monoxygenase and transferase activities in rat by dietary administration of flavonoids. Xenobiotica 19: 1379-1386.

Siess, M.-H., Leclerc, J., Canivenc-Lavier, M.-C., Rat, P., & Suschetet, M. (1995) Heterogenous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. Toxicology and Applied Pharmacology 130: 73-78.

Singhal, R. L. (1995) Quercetin down-regulates signal transduction in human breast carcinoma cells. Biochemical and biophysical research communications 208: 425-431.

Steinmetz, K. A., & Potter, J. D. (1996) Vegetables, fruit, and cancer prevention: A review. Journal of The American Dietetic Association 96: 1027-1039.

Strange, C. R., Jones, P. W., & Fryer, A. A. (2000) Glutathione-S-Transferase: genetics and role in toxicology. Toxicology Letters 112-113: 357-363.

Taipalensuu, J., Tornblom, H., Lindberg, G., Einarsson, C., Sjoqvist, F., Melhuss, H., Garberg, P., Sjostrom, B., Lundgren, B., & Artursson, P. (2001) Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in

normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers.
Journal of Pharmcology and Experimental Therapeutics 299: 164-170.

Takahama, U. (1984) Inhibition of lipoxygenase-dependent lipid peroxidation by
quercetin: mechanism of antioxidant function. Phytochemistry 24: 1443-1446.

Talalay, P. (2000) Chemoprotection against cancer by induction of phase 2 enzymes.
Biofactors 12: p5, 7p.

Talalay, P., De Long, M. J., & Prochaska, H. J. (1988) Identification of a common
chemical signal regulating the induction of enzymes that protect against chemical
carcinogenesis. Proceedings of the National Academy of Sciences of the United States of
America 85: 8261-8265.

Talalay, P., Fahey, J. W., Holtzclaw, D., Prestera, T., & Yuesheng, Z. (1995)
Chemoprotection against cancer by Phase 2 enzyme induction. Toxicology Letters 82/83:
173-179.

Tsyrlov, I. B., Mikhailenko, V. M., & Gelboin, H. V. (1994) Isozyme-and sprecies-
specific susceptibility of cDNA-expressed CYP1A P-450s to different flavonoids.
Biochimica et Biophysica Acta 1205: 325-335.

Tzeng, S. H., Ko, W.-C., Ko, F.-N., & Teng, C.-M. (1991) Inhibition of platelet
aggregation by some flavonoids. Thrombosis Research 64: 91-100.

USDHHS (2000) Healthy People 2010: Understanding and Improving Health. Center for
Disease Control and Prevention National Institutes of Health, Washington, DC.

USDHHS, NIH, & NCI (2001) Cancer Progress Report. USDHHS Public Health Service,
National Institutes of Health, and National Cancer Institute.

Van Duyn, M., & Pivonka, E. (2000) Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. The Journal of The American Dietetic Association 100: 1511-1522.

Walgren, R. A., Lin, J.-T., Kinne, R., K-H, & Walle, T. (2000) Cellular uptake of dietary flavonoid quercetin-4'-B-Glucoside by sodium-dependent glucose transporter SGLT1. Journal of Pharmacology and Experimental Therapy 294: 837-843.

Walle, U. K., French, K. L., Walgren, R. A., & Walle, T. (1999) Transport of genistein-7-glucoside by human intestinal epithelial Caco-2 cells; potential role of MRP2. Research Communications in Molecular Pathology and Pharmacology 103: 45-56.

Wang, H., & Murphy, P. (1996) Isoflavone content in commercial soybean foods. Journal of Agriculture and Food Chemistry 42: 1666-1673.

Wang, H., Tanaka, Y., Han, Z., & Higuchi, C. M. (1995) Proliferative response of mammary glandular tissue to formononetin. Nutrition and Cancer 23: 131-140.

Wang, T. T. Y., Sathyamoorthy, N., & Phang, J. M. (1996) Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis 17: 271-275.

Wargovich, M.J., Eng, V.W., & Newmark, H.L. (1985) Inhibition by plant phenols of benzo{a}pyrene-induced nuclear aberrations in mammalian intestinal cells: a rapid in vivo assessment method. Food and Chemical Toxicology 23(1): 47-49.

Wargovich, MJ., Harris, C., Chen, C. D., Palmer, C., Steele, V.E., Kellof, G.J. (1992) Growth kinetics and chemoprevention of averrant crypts in the rat colon. Journal of Cellular Biochemistry 16F: 51-54.

Warren, A.W., Lupton, J. R., Popovic, N., Hong, M.Y., Taddeo, S.S., Murphy, M.E., Chapkin, R.S., Turner, N.D. (2003) The chemoprevention action of quercetin decreases

the number of high multiplicity abberant crypt foci (ACF), reduces proliferation, and increases apoptosis in rat colonocytes. The FESEB Journal 17: A1202.

Wattenberg, L. (1992) Inhibition of carcinogenesis by minor dietary constituents. Cancer Research 52 (suppl): 2085s-2091.

WCR, and AICR (1997) Food, nutrition and the prevention of cancer: A global perspective. World Cancer Research Fund and American Institute for Cancer Research, pp. 55-421.

Wei, H., Bowen, R., Cai, Q., Barnes, S., & Wang, Y. (1995) Antioxidant and antipromotional effects of the soybean isoflavone genistein. Society for Experimental Biology and Medicine 208: 124-130.

Welshons, W. V., Rottinghaus, G. E., Nonneman, D. J., M, D.-T., & Ross, P. F. (1990) A senstivie bioassay for detection of dietary estrogens in animal feeds. Journal of Veterinary Diagnostic Investigation 2: 268-273.

Whitaker, L.M., Kelloff, G.J., Steele,V.E., & Lubert, R.A. (1996) Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz{a}anthracene-induced mammary cancer in rats.   Carcinogenesis 17: 1305-1311.

Whitlock, J. P. (1999) Induction of cytochrome P4501A1. Annual Review of Pharmacology and Toxicology 39: 103-125.

Willett, W. C. (2000) Diet and Cancer. The Oncologist 5: 393-404.

Williams, P. L., James, R. C., & Roberts, S. M. (2000) Principles of toxicology: environmental and industrial applications. John Wiley and Sons Inc., New York, pages 1-603.

Williamson, G., Day, A. J., Plumb, G. W., & Couteau, D. (2000) Human metabolic pathways of dietary flavonoids and cinnamates. Biochemical Society Transactions 28: 16-21.

Wolffram, S., Block, M., & Ader, P. (2002) Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. Journal of Nutrition 132: 630-635.

Xu, X., Wang, H. J., Murphy, P. A., Cook, L., & Hendrich, S. (1994) Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. Journal of Nutrition 124: 825-832.

Yang, C. S., Landau, J. M., Huang, M.-T., & Newmark, H. L. (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual Review of Nutrition 21: 381-406.

Yannai, S., Day, A. J., Williamson, G., & Rhodes, M. J. C. (1998) Characterization of flavonoids as monofunctional or bifunctional inducers of quinone reductase in murine hepatoma cell lines. Food Chemical Toxicology 36: 623-630.

Yasuda, T., Mizunuma, S., Kano, Y., Saito, K.-I., & Ohsawa, K. (1996) Urinary and biliary metabolites of genistein in rats. Biological and Pharmaceutical Bulletin 19: 413-417.

Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K., Fujioka, A., Nishino, H., & Aoike, A. (1990) The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Lett 260: 10-13.

You, W.-C., Blot, W. J., Chang, Y.-S., Ershow, A. G., Yang, Z.-T., An, Q., Henderson, B., Xu, G.-W., Fraumeni, J. F., & Wang, T.-G. (1988) Diet and high risk of stomach cancer in Shandong, China. Cancer Research 48: 3518-3523.

Zhang, Y., Hendrich, S., & Murphy, P. A. (2003) Glucuronides are the main isoflavone metabolites in women. Journal of Nutrition 133: 399-404.