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thesis entitled

Consumption of Black Beans and Navy Beans Reduced Azoxymethane Induced Colon Cancer in Rats

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Human Nutrition

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Date March 29, 2001

CONSUMPTION OF BLACK BEANS AND NAVY BEANS REDUCED AZOXYMETHANE INDUCED COLON CANCER IN RATS.

By

Laura Hangen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

2001

ABSTRACT

CONSUMPTION OF BLACK BEANS AND NAVY BEANS REDUCED AZOXYMETHANE INDUCED COLON CANCER IN RATS.

By

Laura Hangen

Dry beans are an important food staple in the traditional diet of many countries throughout the world. Epidemiological studies have shown that in many Latin American countries, where consumption of beans is high, the incidence of colon cancer is low. Beans are a very nutrient dense food and are good sources of dietary fiber, protein and many phytochemicals that have shown anticarcinogenic properties. The objective of this study was to determine if consumption of black beans (BB) and/or navy beans (NB) would reduce colon carcinogenesis in rats. Rats (21 days old) were separated into three groups and each group was fed a different diet: modified AIN 93G diet (control), BB or NB. Initially the beans were soaked over night and cooked until they were soft. Later they were dried in a convection oven (50-60° C) and then ground. All diets were formulated to provide the same amount of digestible protein (12.69%) and fat (16%). The bean diets were supplemented with sulfur amino acids to insure adequate protein quality. The rats were fed a total of 36 weeks. After the first month, the rats were injected with the carcinogen azoxymethane (AOM). Thirty weeks later the animals were sacrificed and the colons were removed. All abnormal appearing tissue in the colon was examined histologically. The incidence of tumors in the rats fed the BB (28%) and the NB diets (25%) was significantly lower than in rats fed the control diet (61%). Tumor multiplicity was also significantly lower in the bean diets (BB 1.11 and NB 1.0) than in the control diet (2.2). The average weight of tumors in the control group (0.16) was heavier than the tumors in either bean diet (BB 0.08 and NB 0.07). However, these differences were not statistically significant. We concluded that both types of bean diets possess anticarcinogenic properties and that they were able to reduce the incidence of tumors in AOM induced colon cancer in rats.

DEDICATION

Una vez concluida my tesis quiero pedir gracias a Dios por guiarme en esta aventura que nunca imagine poder lograr. Tambien quiero agradecer a mis padres por el apoyo emocional y por estar siempre conmigo cuando lo necesite. Quiero agradecer a mis hermanos por apoyarme cada instante del camino y por ser mis amigos y confidentes. Tambien quiero agradecer a Eduardo por estar conmigo, por amarme y por aconsejarme siempre. Quiero que sepan que esto y mas lo hago por ustedes porque los adoro y no podria vivir sin ustedes.

A todos mis amigos que me apoyaron y estuvieron junto a mi apesar de la distancia les agradezco miles.

ACKNOWLEDGEMENTS

I want to thank Dr Bennink for his help and guidance throughout my study and for giving me the opportunity to further my education. To my committee members for their input and review of my thesis. I also want to thank my lab mates, especially Elizabeth Rondini, for their help and constant encouragement.

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I. INTRODUCTION

Colon cancer is one of the most common cancers in the developed countries. Colon cancer is considered the third leading cause of death due to cancer for both men and women in the United States (Greenlee et al 2000). Many risk factors have been studied and associated with this type of cancer. These risk factors are classified into two major groups, environmental factors and genetic factors. It is estimated that genetic factors are responsible for causing between 5 to 10% of all cancers, but these factors cannot be modified (Cohen et al 1995). Many environmental factors such as diet, smoking, stress, chemical pollutants and drugs increase the risk of developing cancer (Doll and Peto 1981).

Epidemiological and experimental studies have shown that diet plays an important roll in the development of colon cancer (Lee et al 1989). Data from many countries show that colon cancer is generally lower in countries like Africa and Latin America than in Australia and North America. This geographical distribution has been strongly correlated with differences in nutritional patterns. Countries that present a higher incidence of colon cancer have a higher consumption of fat and meat and a lower consumption of fruits, vegetables and legumes.

Legumes are good sources of dietary fiber, folate and many phytochemicals associated with a lower incidence of colon carcinogenesis. Epidemiological data show that countries that consume dry beans (phaseolus vulgaris L.) in their regular diet present lower incidence of cancers like colon cancer (Correa, 1981). Hughes et al (1997) also showed that feeding pinto beans reduced chemically induced colon carcinogenesis in rats.

Phytochemicals (non-nutrient, plant compounds with biological activity) in beans that could reduce colon cancer include saponins, phenolic compounds and phytic acid. Dietary fiber and resistant starch may lower the incidence of colon cancer because they are fermented in the colon to produce short chain fatty acids (SCFA). Butyrate, one of the SCFA, is considered to protect against colon cancer.

Even though the evidence that dry bean consumption would lower the risk of colon cancer is very limited, beans contain several components that have been associated with a lower incidence of colon cancer. Therefore, the objective of this study is to determine if eating beans will reduce carcinogenesis in rats.

II. BACKGROUND

A. REVIEW OF COLON CANCER EPIDEMIOLOGY

Overall, cancer is considered the second leading cause of death in the United States after coronary heart disease. There are many types of cancers, but the greatest incidences are observed in lungs, prostate/breast and colon/rectum cancers. In the year 2000 the U.S. cancer statistics reported 1,220,100 new cases of cancer in the United States and approximately 93,000 of those new cases were colon cancers (Greenlee et al 2000). Even though colon cancer incidence has decreased significantly between 1990 and 1996, it is responsible for 11% of all cancer deaths and is considered the third leading cause of death within all cancers for both sexes and all ages (Greenlee et al 2000).

Worldwide data confirm that colon cancer incidence has a specific geographic distribution, having low incidences in countries like Asia, Africa and Latin America and high incidence in industrialized countries like Australia and North America (IARC, 1988; Cohen et al 1995). The variation in incidence of colon cancer between one part of the world and another can reach up to 20 fold or more. This geographical distribution has been strongly correlated with the differences in environmental factors especially in nutritional patterns between these countries.

On the other hand, several migration studies have demonstrated that people who have moved to a new location experience a shift (increase or decrease) in their risk of developing cancers, such as colon cancer, between their country and their host country. In 1956, Smith developed one of the first migration studies of Japanese who moved to the

U.S., and he demonstrated that the rates of colon carcinomas among US-Japanese were lower than U.S. Americans and higher than Japanese who lived in Japan (Smith, 1956). Many other studies have confirmed this hypothesis and have demonstrated that environmental factors are very important in the development of colon cancer (Wanebo, 1993; Ruddon 1995).

B. CARCINOGENESIS

Cancer is a disease, in which cells multiply out of control, forming masses that disrupt normal functioning of one or more organs. Cancer fundamentally involves damage to the structure and function of the cell's DNA (Ames, 1989; Sporn, 1991; Wanebo 1993; Ruddon, 1995; Chang et al 1997). These mutations are transmitted to the daughter cells, which in turn, progress to malignancy (cancer cells capable of metastasis). Cancer usually develops in cells that are constantly replicating, such as the gastrointestinal tract. These types of cells are more vulnerable to errors and mutations because they are constantly dividing and have less time for DNA repair, causing the mutation to become fixed (Lipkin, 1974; Creasey, 1985; Lipkin, 1988; Ames, 1989; Ruddon, 1995).

In normal colonic cells, mitosis is a very controlled process in which phosphorylation and dephosphorylation of specific proteins within the cell controls transcription, translation, proliferation or differentiation (Norbury and Nurse 1992; Lewin, 1994). Normally colon crypts have stems cells, which divide in the first half of the crypt. As the cell travels toward the top of the crypt, it undergoes terminal

differentiation and the cell cannot go through the process of division any longer. When the cell reaches the top of the crypt, it undergoes apoptosis and exfoliates (Norbury and Nurse 1992; Lewin, 1994). Persons that have an increase in cell proliferation and a decrease in cell differentiation and apoptosis in the colon mucosa have an increased risk of colon cancer. The altered cell cycle favors an accumulation of cells harboring mutations that eventually will cause formation of adenocarcinomas (Lipkin, 1974; Lipkin, 1988).

The prevailing theory of development of cancer indicates that this process may be divided into three main stages: initiation, promotion, and progression (Fearon and Vogelstein 1990; Ruddon, 1995). Initiation is the stage where the cells are exposed briefly to a potent carcinogen. This event causes mutations in the genetic material (DNA) of the cell. If the cell divides before the mutation is repaired, the mutation is passed on to the daughter cells. The promotion stage is a slow and gradual process where the initiated cell needs a prolonged exposure to the promoting agent in order to develop malignancy. In this step the cell enters a proliferation state that propagates the initiated damage and produces clones of the altered cell. During this stage, growth of initiated tumor cells is stimulated by increasing proliferation of cells and by decreasing differentiation and apoptosis (programmed cell death) in cells. But even though the cells are mutated and proliferating, at this level the process is reversible through anti-carcinogenic agents which are capable of stopping this process. The progression stage occurs when the clonal proliferation of altered cells continues and the cells lose growth control. This process is irreversible because of the pronounced changes in the genome. At this level, the cells acquire a high resistance to cytolytic T lymphocytes and develop invasive and metastic

characteristics. During this stage, the altered cells progress to clinically detectable tumors (Ruddon, 1995).

B.1 Colon cancer development

Colon cancer arises as an accumulation of genetic changes in single cells, which are responsible for altering the balance between cell proliferation, differentiation and apoptosis (Lipkin and Deschner 1976; Lewin, 1994; Chang et al 1997; Staunton and Gaffney 1998; Winawer, 1999). These alterations are usually activation of cellular oncogenes through mutation, rearrangement or amplification of the genes, or through inactivation of tumor suppressor genes by deletion or mutation (Creasey, 1985; Vogelstein et al 1988; Fearon and Vogelstein 1990; Vogelstein and Kinzler 1993). This theory is based on the idea that genetic alterations in human colorectal tumors increase as the disease progresses clinically. It has been stipulated that most colon cancers develop from pre-existing benign adenomas. Fearon and Vogelstein (1990), proposed a genetic model for colorectal tumorgenesis, in which they described the genetic alterations and the sequence of the alterations in this process. This model has three main characteristics: first, the cancer develops as a result of activation of oncogenes plus inactivation of tumor suppressor genes; second, to obtain malignant tumors at least four or five alterations have to be present; and finally, the formation of the tumors have a preferred sequence of genetic alterations. However, the amount of changes rather than their order determines tumor development (Vogelstein et al 1988; Fearon and Vogelstein 1990; Vogelstein and Kinzler 1993). The proposed model involves at least four genetic events: mutation or deletion of adenomatous polyposis coli gene (APC) on chromosome 5q, mutation of *ras*

genes on chromosome 12p, deletion of "deleted in colorectal carcinoma" gene (DCC) on chromosome 18q and deletion of p53 gene on chromosome 17p (Fearon and Vogelstein 1990).

Loss of specific regions in chromosomes occurs frequently in colon cancer. These allelic losses demonstrate that the lost genes have tumor suppressor activity that regulates growth and differentiation in cells. One of the first chromosome losses in the progression of colon cancer is the APC, which is located in the chromosome 5q (5q21) (Bodmer et al 1987; Fearon and Vogelstein 1990). The APC protein is involved with cell maturation, therefore when the APC protein is mutated the cells continue to proliferate and no differentiation or apoptosis is observed (Morin et al 1996). This allelic loss has been observed in 20-50% of colon carcinomas and in 30% of adenomas (Vogelstein et al 1988; Sasaki et al 1989). However, there are some individuals that suffer familial adenomatous polyposis (FAP), which is a condition that presents mutations and not a deletion of the same gene. FAP is an inherited predisposition to colon carcinoma that gives rise to multiple adenomatous polyps in the colon, which have a high probability of progressing to malignancy (Bodmer et al 1987; Solomon et al 1987; Fearon and Vogelstein 1990; Bodmer, 1999).

Some of the most prominent and frequent alterations in oncogenes are in the *ras* gene family (*c-Ha-ras, c-Ki-ras* and *N-ras*) on chromosome 12p. *Ki-ras* mutations are the most frequent *ras* mutation in colon cancer (Forrester et al 1987; Bos et al 1987; Fearon and Vogelstein 1990; Mclellan et al 1993). These genes encode GTP binding proteins, which are located on the inner surface of the plasma membrane and their function is associated with stimulus of cell proliferation and GTPase activity. Several studies show

that approximately 50% of all carcinomas and adenomas larger than one centimeter have mutations in the *ras* gene (Bos et al 1987; Forrester et al 1987). These mutations are considered one of the initiating events in tumor development and it is estimated that adenomas that have these mutations are more likely to progress to carcinomas (Fearon and Vogelstein 1990; Khosravi-far and Der 1994). Other oncogenes involved in this process are *c-myc, c-myb, neu* and *trk*, but the role of these oncogenes in the development of colon cancer are less clear.

The deletion of a region in chromosome 18q is also very frequent in colon cancer and is estimated that approximately 70% of colon carcinomas and 50% of adenomas have lost this region (Vogelstein et al 1988; Fearon and Vogelstein 1990). The region deleted in this chromosome contains the tumor suppressor gene known as DCC, which encodes a protein whose functions are related to cell adhesion, cell to cell communication and normal state of cell differentiation (Fearon et al 1990). More allelic losses have been reported in several colon tumors, including regions of chromosomes 1q, 4p, 6q, 8p, 9q and 22q. However the frequency of these deletions is much lower.

Another region of chromosomes that is commonly deleted in colon carcinomas is located in chromosome 17p, a region that encodes for gene p53 (Fearon and Vogelstein 1990). The allelic loss of this region is associated with the progression of adenomas into carcinomas, which indicates that this is a late event in the progression to malignancy. It is estimated that approximately 75% of all carcinomas present this deletion (Vogelstein et al 1988). Studies show that most tumors have deletion of one of the p53 alleles and mutations in the other allele, which suggest inactivation of the tumor suppressor activity of the gene. The p53 gene encodes a protein that stops cells with damaged DNA from

passing from the G1 phase to the S phase in the cell cycle. When the p53 gene is mutated or deleted, the cell has unrestrained proliferation that can lead to development of carcinomas (Vogelstein et al 1988; Baker, et al 1989; Fearon and Vogelstein 1990; Cousin et al 2000).

In general, the data show that even though there is a specific sequence of mutations and deletions of genes in colon cancer development, it is the accumulation of genetic alterations that really determines the development of colon cancer (Vogelstein et al 1988; Fearon and Vogelstein 1990; Vogelstein and Kinzler 1993).

B.2 β-catenin

Recently, scientists have begun to elucidate the role of β-catenin in the development of colon cancer. β-catenin is a protein associated with cell adhesion and cell communication because it forms part of the cell adhesion molecules (CAMs). E-cadherins are involved in the adhere junction in epithelial cells and intracellularly E-cadherin binds to β-catenin, which connects to α-catenin. This complex is attached to the actin cytoskeleton. The dissociation of this complex occurs when β-catenin is phosphorylated or when growth factors interact, allowing the cell to migrate. This is an important process in tissue healing and in carcinogenesis (Ilyas and Tomlinson 1997; Morin, 1999).

β-catenin is also found as a free monomer at a low concentration in the cytoplasm. Normally APC protein regulates the concentrations of β-catenin by directing β-catenin degradation to avoid accumulation of this component. However, when the concentrations of β-catenin increase, it can translocate into the nucleus and increase

transcription, affecting genes associated with cell cycle control and apoptosis (Bratbletz et al 1998; Ben-Zeéz and Geiger 1989; Morin, 1999).

In colon carcinogenesis the APC gene is one of the most common mutations observed, therefore when the APC protein is not available for the degradation of β-catenin, this component enters the nucleus and can stimulate carcinogenesis (Bratbletz et al 1998; Ilyas and Tomlinson 1997).

B.3 Risk factors

Risk factors associated with colon cancer development are divided into environmental factors and genetic factors (Jacobs, 1988; Newberne and Conner 1988).

B.3.1 Genetic factors

It has been estimated that genetic factors are responsible for causing between 5 to 10% of all cancers (Cohen et al 1995). In colon cancer, heredity plays an important role in many situations, because there are many disorders that predispose to different degrees of malignancy (Ruddon, 1995). One of the most common disorders is the familial adenomatous polyposis (FAP) and the persons that have these conditions are at a very high risk of developing colon carcinomas, unless they undergo a total colostomy. Some of the other disorders that have an inherited predisposition to colon cancer are: Gardner's syndrome, juvenile polyposis and Turcot's syndrome.

B.3.2 Environmental factors

Scientists have identified many environmental factors associated with the development of colon cancer and the most relevant factors are: diet, smoking, physical inactivity, stress, chemical pollutants and drugs. Even though there are many environmental components that could promote the development of cancer, several nutritional factors have shown a strong correlation with carcinogenesis (Drasar and Irving 1973; Doll and Peto 1981; Newberne and Conner 1988; Boutwell, 1988; Slattery et al 1998; Shike, 1999). Doll and Peto estimated that nutritional factors were responsible for approximately 35% of all cancer mortality (Doll and Peto 1981). Further studies have shown that colon cancer is the form of cancer that is the most strongly associated with diet (Drasar and Irving 1973).

Scientists have undertaken many studies with an attempt to identify the components within the diet that promote or protect against colon cancer. Several types of foods and nutrients have been studied, but only some of these have been associated with colon cancer incidence. Epidemiological and experimental studies have shown that the risk of colon cancer is increased in populations that have a high fat and high meat diet, such as the Jewish population of New York who have a high incidence of colon cancer. Most dietary fat in western diets comes from meat, which is the reason that the incidence of colon cancer is correlated not only to fat but also to animal protein. A meta-analysis conducted by Potter et al in 1993, showed that out of the thirteen well controlled studies, eleven were able to identify a strong correlation between meat and fat consumption and colon cancer incidence (Potter et al 1993). The proposed mechanism by which high intakes of fat cause colon cancer development is based on the fact that consumption of fat

promotes secretion of bile acids, which can irritate the colon mucosa. When bile acids are exposed to intestinal bacteria they are transformed into cytotoxic secondary bile acids (lithocholic and deoxycholic) by the enzyme 7-α dehydroxylase. The production of secondary bile acids are known to be cytotoxic to the colon and can act as tumor promoters, increasing cell proliferation in the colon which favors colon cancer development. It has also been shown that increased concentrations of all fecal bile acids and cholesterol are associated with increased risk of colon cancer. Some studies show that the type of fat may also be important in colon cancer risk. Saturated fatty acids show a stronger correlation to colon cancer risk than polyunsaturated fatty acids or total dietary fat. In general, dietary fat is one of the environmental factors that have sufficient data to demonstrate a positive correlation with colon carcinogenesis.

On the other hand, the risk of colon cancer is decreased when the diet is high in fruit, vegetables, cereals and legumes, or when food restriction is observed (Shike, 1999). In an effort to prevent this type of cancer, the recent tendency is to attempt to identify which foods, and even which components within those groups have anticancer properties. Fruits and vegetables have been studied together because of their origin and their similarities in composition. Many studies have examined different fruits and vegetables, and the data show that fruit and vegetables are the main food groups that protect against colon cancer (Ames, 1989; Lee et al 1989; Potter et al 1993; Kaaks and Riboli 1995). The most consistent observations are with vegetable intake, which show a strong inverse association with colon cancer, especially for cruciferous and green vegetable consumption. The meta-analysis by Potter et al (1993), showed an inverse association in twenty-three out of the twenty-eight studies that looked at vegetables and colon cancer

incidence. The inverse correlation with fruits is less consistant. In general, fruits and vegetables are considered good sources of fiber and many other plant components called phytochemicals, which have been linked to reduced cancer risk. The protective properties of dietary fiber against colon cancer have been studied since the 1970s, when scientists started to look at African populations, who had high intakes of dietary fiber and a low incidence of colon cancer. However, even though many studies of fiber and colon cancer have been developed, the conclusions are still controversial because many studies show an inverse association but others haven't found any protective effect. In the meta-analysis elaborated by Potter et al (1993), only ten out of the sixteen main studies related to dietary fiber and colon cancer were able to show a protective effect of fiber (Potter, 1993; Kaaks and Riboli 1995). Dietary fiber is responsible for increasing stool bulk and decreasing transit time (Harris and Fergurson, 1993). This may account for the protective effect of fiber because it decreases the contact of promoters with the colon mucosa by diluting the intestinal content and/or by decreasing time of exposure to the promoter. Fiber is fermented by colonic bacteria, which can protect the colon by producing short chain fatty acids (SCFA) and by inhibiting formation of secondary bile acids, which are toxic to epithelium (Van Soest, 1978; Bringham, 1987). The relationship between dietary fiber and colon cancer is equivocal, but many studies do show a protective effect.

The third food group associated with colon cancer incidence is cereals or whole grains. Dietary guidelines recommend the consumption of whole grains to prevent chronic diseases such as colon cancer. Epidemiological studies support the theory that whole grains as well as fruits and vegetables, protect against colon cancer (Alberts, et al 2000). Whole grains are important sources of dietary fiber, several vitamins and trace

minerals and many phytochemicals (phenolic acids, phytic acid, tannins), which may be important in prevention of colon cancer (Harris and Fergurson 1993; Kristchevsky, 1999; Slavin et al 1999). When the grains undergo the milling process they lose most of the bran and germ, which contains most of the fiber, and much of the vitamins, minerals and phytochemicals of the grains. Several studies have demonstrated that wheat bran is one of the most effective fibers in protecting against colon tumor development (Zoran et al 1997; Alberts, et al 2000). Some of the mechanisms proposed are based on the fact that dietary fiber has the ability of decreasing transit time and diluting intestinal contents, which decreases the time of exposure of promotors to the colon epithelium. Also, fiber is fermented by colonic bacteria to produce SCFA that may protect against colon cancer (Kristchevsky, 1999; Slavin et al 1999; Compher et al 1999; Earnest et al 1999). The antioxidant properties of many of the components within the grains are proposed to reduce oxidative damage, which could reduce colon carcinogenesis.

The last food group is the legumes. Recently there has been a great interest in legumes, which are good sources of dietary fiber and many phytochemicals associated with a reduced incidence of cancer. On the other hand, legumes are good sources of protein and many essential vitamins and minerals, and are very low in fat. Also, limited epidemiological data show that countries that consume legumes in their regular diet present lower incidence of cancers like colon cancer (Correa, 1981; Steinmetz and Potter, 1991). Legumes, such as soybeans and dry beans, are one of the main sources of protein and fiber in many developing countries, in part because of their low cost and availability. Currently, legumes such as soybeans have been associated with a lower incidence of colon cancer, but legumes such as soybean are not widely consumed by a large section of

the world population, so the benefit is obtained by fewer people. On the other hand, dry beans are widely consumed in countries in Central and South America where colon cancer incidence is low.

Another important factor associated with colon cancer development is caloric restriction (Lee et al 1999). This factor has been studied since the 1940s, when Tannenbaum demonstrated that food restriction could reduce tumor incidence in mice (Tannenbaum, 1945). However, recently scientists wanted to determine if calorie intake and dietary fat had independent effects on colon cancer risk. Kumar et al (1990) published one of the first studies, where they fed high fat and low fat diets to male rats and they also had calorie restriction of 10, 20 and 30%. The results showed there was a significant reduction in incidence and multiplicity of tumors in the rats that had 20 and 30% caloric restriction even though the diets had a high percentage of calories from fat. This suggests an independent role for fat (Kumar et al 1990). Epidemiological studies show that increased calorie intake is associated with an increase in colon cancer risk. However, the connection between calorie intake, energy balance and body weight is not considered in many of the studies, which makes interpretation of the results more difficult. The hypothesis for how caloric restriction can prevent colon carcinogenesis is by causing increased biosynthesis and turnover of protein and decreased macromolecular damage within the cells (Lee et al 1999).

Several vitamins and minerals have been associated with colon cancer development. Calcium is one of the minerals that have shown protection against colon carcinogenesis. Several epidemiological, experimental and clinical trials have shown that supplementation with calcium lowers the risk of developing colon cancer, because

calcium decreases proliferation and increases differentiation (Kleibeuker et al 1995). The suggested mechanism is that calcium combines with fatty acids and bile acids to form salts that precipitate, minimizing contact between the epithelium and cytotoxic compounds. Data also suggest that calcium acts directly on the colonic cells causing decreased proliferation and increased differentiation through intracellular mechanisms (Kleibeuker et al 1995). Another mineral involved in colon carcinogenesis is selenium. Epidemiological studies show increased incidence of colon cancer in areas where the concentration of selenium in crops and soil is low (Fleet and Mayer 1997). Animal studies also demonstrate that supplementation of diets with selenium can reduce the incidence of colon cancer up to 50%. Data suggest that selenium has antioxidant properties and many other metabolic functions such as stimulation of apoptosis and through its role in glutathione peroxidase (Russo et al 1997; Fleet and Mayer 1997). Only a few studies show any correlation between vitamins and risk of colon cancer. There is a possible protection against colon cancer with the carotenoids, but the results are controversial. Some epidemiological studies and experimental studies show an inverse correlation with carotenoids and colon cancer, however other studies show no correlation. In the case of vitamin E, C, D, and folate, the number of studies that show a protective effect are insufficient to establish a protective role.

C. DRY BEANS (*PHASEOLUS VULGARIS*)

Dry beans are categorized as legumes since they have the characteristic of seed bearing pods. Legumes are divided into two major groups, the oily beans (soybean, peanuts) that are important for their protein, dietary fiber and oil content. The grain beans (dry beans, lentils, garbanzos) are important for their protein, dietary fiber and starch content. Any bean with very low fat content that precludes oil extraction is considered grain bean (Messina, 1999). The interest in studying dry beans arises from the fact that beans, as well as fruits and vegetables, contain dietary fiber and several phytochemical components that have been linked to a reduction in the incidence of colon cancer. Some of these components include protease inhibitors, polyphenolic compounds, saponins, phytic acids, and resistant starch.

There are approximately 100 different market classes of beans that are cultivated around the world. Some of the most common bean market classes produced and consumed around the world are: pinto beans, navy beans and black beans. India, China and Latin America consume legumes such as dry beans in their regular diet. The consumption of dry beans in the United States is low (8lbs. per capita), even though it produces almost one third of the world supply.

C.1 Composition of dry beans

Dry beans are considered an excellent food, not only because its nutrient composition but also because it is an inexpensive and widely available product. Beans are an economical source of concentrated protein, a rich source of complex carbohydrate

(starch and dietary fiber), a good source of many vitamins and minerals, and a variety of phytochemicals. For the purpose of this thesis dietary fiber is defined as: "the skeletal remains of plant cell walls incapable of digestion by human digestive enzymes" (Trowell, 1976). Under this definition resistant starch and oligosacharides are not considered dietary fiber.

Even though the market classes of dry beans vary in color, size, shape and flavor, the nutritional composition is very similar. The energy obtained by consuming beans comes mainly from starch and protein, which are the main components of the beans. Carbohydrates provide 60 to 65% of the beans nutrients. The main carbohydrate is starch although a small amount of monosaccharides and disaccharides are present. Oligosaccharides such as raffinose, stachyose and verbascose are also present in the beans. These oligosaccharides are not digested in the small intestine because humans don't have the necessary α-galactosidase; therefore, they pass to the colon where they are fermented by colonic bacteria. Dry beans are also a good sources of insoluble and soluble fiber (Tovar, 1994, Lintas et al 1995). Beans are a concentrated source of protein, however protease inhibitors, lectins, tannins and dietary fiber lower the digestibility of the protein and limits its digestion and absorption. The fat content of beans is very low (0.8-1.5%) and unsaturated fatty acids predominate. The vitamins present in dry beans are mainly water-soluble vitamins, especially thiamin, riboflavin, niacin and folate. The fat-soluble vitamins are present in very low amounts because of the low fat content. Several types of minerals are present in the beans, such as calcium, iron, copper, zinc, phosphorus, potassium and magnesium. However, bioavailability of minerals from plant

origins is lower than from animal sources. Table 1 shows the nutrient composition of different classes of legumes.

Table 1.

Nutrient content of selected legumes (Per 100 g of edible portion)*

LEGUME	CHO+ (%)	PROTEIN (%)	FAT (%)	FIBER (g)	Folate (ug)
Black bean	47.2	21.1	1.4	15.2	444.3
Navy bean	36.2	22.3	1.3	24.4	369.7
Soybean	20.9	36.5	19.9	9.3	375.1

*Adapted from USDA Nutrient database for standard reference. Release 13. 1999.

+ Non fiber carbohydrates (CHO)

C.1.1 Protein

Dry beans are a very good source of protein and it's estimated that they contain approximately 21 to 25% of crude protein. Even though beans are a good source of protein with high amounts of essential amino acids such as lysine, they are deficient in sulfur amino acids such as methionine. This is why beans should be consumed with cereal grains (e.g. rice or corn), which are adequate in sulfur amino acids but low in lysine. When two thirds of the protein is derived from cereal grains and one third from legumes the mixture forms a high quality protein food, which is adequate for all people ten years and older.

One of the main problems with the protein in the beans is its low digestibility. Bressani et al (1982) demonstrated that navy bean had the highest protein digestibility compared to red and black beans (74.1, 68.4 and 66.7% respectively). The low protein digestibility is due to protease (trypsin and chymotrypsin) inhibitors, tannins, high amounts of dietary fiber, lectins and the physical structure of the protein that resists digestion (Leaky, 1995). The protease inhibitors retard bean protein digestion by complexing with the corresponding enzymes (trypsin and chymotrypsin), which inactivates the enzymes and stops protein digestion. Blanco et al (1986) demonstrated that when beans are cooked, trypsin inhibitor activity is reduced by 28 to 73%, and protease inhibition by tannins is reduced by 9 to 72%. Thus, cooking makes the protein more digestible. Also, they showed that even though the pinto beans have more antinutritional factors than navy and black beans, protein digestibility was similar (Blanco et al 1986). Another factor that could limit the digestion of the protein is the physical structure of the protein itself. Many foods need grinding and cooking to break the cell wall release protein and starch. For example, beans are consumed whole and mastication is not adequate to break all cells. Therefore, many cell walls remain intact and the protein is incompletely digested because the proteins are not accessible to proteolytic enzymes in the gut. The amount of dietary fiber, tannins and lectin in the diet can limit the digestion and absorption of the proteins too. When the amounts of these components in the diet increase, the digestibility of protein tends to decrease, because fiber, tannins and lectin associate with the protein and decrease digestive enzyme accessibility to protein (Bressani et al 1991).

C.1.2 Dietary fiber and resistant starch

Dietary fiber as defined previously includes cellulose, hemicelluloses, lignin, pectin, and gums. On the other hand, recent studies have shown that a portion of the starch consumed, escapes digestion in the small intestine and this fraction is known as resistant starch (RS). Several scientists have suggested that RS should be included in the dietary fiber, based on the fact that RS as well as dietary fiber escapes digestion in the small intestine. However, this decision could cause confusion in recognizing dietary fiber, because the amounts of RS entering the colon are very inconsistent and can vary depending on cooking process and temperature, among other factors. Therefore, RS will be considered as an independent component in this review.

C.1.2.1 Classification of dietary fiber

Dietary fiber is divided into two main groups: the soluble and the insoluble fibers. Cellulose, hemicelluloses and lignin are the main components known as insoluble fiber. Insoluble fibers are found in fruits and vegetables but larger amounts are found in whole grain cereals. Pectin, gums and mucilage are the soluble fibers, and are present in fruits, vegetables, oat bran and legumes (Van Soest, 1978; Bringham, 1987; Asp, 1995).

Dry beans are excellent sources of dietary fiber. Beans contain significant amounts of insoluble and soluble fibers. It is estimated that the amount of total fiber in cooked beans ranges between 15 and 25g/100g of beans (USDA, 1999). Dietary fiber provides many benefits to the digestive tract. Fiber increases stool bulk, decreases intestinal transit time, is fermented by colonic bacteria to produce SCFA and may protect the colon against diseases such as colon cancer (Asp, 1995).

On the other hand, RS is composed of starch that escapes digestion in the small intestine for several reasons. There are three types of RS: RS1 caused by physical entrapment of the starch granule within a food, which prevents the maltase from reaching the starch (e.g. Coarsely ground grains); RS2 is related to the structure of the granule. RS2 has a crystalline structure that makes it more resistant to enzymes (ex. Non-gelatinized amylose in corn starch); and RS3 caused by retrogradation through food processing (e.g. cooked and cooled potato). It is estimated that approximately 8 to 40g (10% of starch in diet) of the carbohydrates consumed in the diet reaches the colon every day. The precise amount of RS from beans that reaches the colon is not known. However, it is estimated that the amount of RS delivered to the colon is significant (Noah et al 1998).

C.1.2.2 Fermentation of dietary fiber and resistant starch

Fermentation is defined as the anaerobic breakdown of carbohydrates and proteins by bacteria. The colon contains more than 400 different species of bacteria that are capable of fermentation. Dietary fiber is the principal substrate for fermentation by colonic bacteria, however resistant starch, indigestible proteins and oligosacharides are also fermented. When fermentation occurs, the main end products are SCFA, water, methane, hydrogen and carbon dioxide (Ehle et al 1982; Cummings and Macfarlane 1991; Rombeau and Kripke 1991; Scheppach et al 1995; Cummings et al 1996; Fergurson et al 2000).

The SCFA produced during fermentation are the C2-5 organic fatty acids, especially acetate, propionate and butyrate, which accounts for approximately 83% of the

fatty acids produced. The molar ratio for SCFA in humans are approximately 55:18:11:6 (acetate:propionate:butyrate:other acids) (Cummings et al 1987; Velazquez et al 1996). However, dietary substrates delivered to the colon influences which SCFA will be produced in greater amounts. For example, when starch is available the bacteria produce more butyrate and when pectin (more oxidized) is available more acetate is produced.

Cummings and Macfarlane (1991) demonstrated that approximately 95% of the SCFA produced in the colon are absorbed during transit through the colon. The absorption of SCFA is very rapid and it is concentration dependent, i.e. higher concentrations lead to greater amounts being absorbed (Cummings and Macfarlane 1991; Velazquez et al 1996).

The mechanism of SCFA absorption have been studied and two main mechanisms have been described. In the proximal colon, the main mechanism of absorption is thought to be through passive diffusion, because the SCFA are lipid soluble. However, a source of hydrogen ions is necessary because the SCFA are present in the anionic form. The hydrogen ions are obtained via sodium/hydrogen exchange in the apical membrane of the colonocytes. The second mechanism is through ion exchange with HCO_3. The carbonic anhydrase provides the hydrogen ions and the HCO_3 necessary for both mechanisms (Binder and Mehta 1989; Engelhardt, 1995; Velazquez et al 1996; Klein et al 1998).

At the distal colon passive diffusion is more important than in the proximal colon, however, the hydrogen ions are obtained from the potassium/hydrogen ATPase exchange at the apical membrane. The ion exchange with HCO_3 and SCFA is also present in the distal colon (Engelhardt, 1995; Velazquez et al 1996; Klein et al 1998).

When the SCFA are absorbed they may be utilized by the colonocyte for energy or they may pass into the blood system and travel to the liver where they are cleared rapidly for further metabolism (Cummings and Macfarlane 1991; Klein et al 1998).

Studies estimate that 60-70% of the energy needed by the colonocytes is obtained from SCFA, with butyrate the primary metabolic fuel. Propionate can be a precursor of gluconeogenesis. Also, propionate may lower serum cholesterol. This effect could be due to inhibition of hepatic cholesterol synthesis or by increased uptake of cholesterol by the liver. On the other hand, acetate is present in peripheral tissues where it is oxidized and becomes an important fuel for these tissues. Cardiac and skeletal muscle, as well as the brain can metabolize acetate to obtain energy. Acetate is also absorbed by liver and used for ketogenesis and fatty acid synthesis (Cummings et al 1987; Cummings and Macfarlane 1991; Velazquez et al 1996).

The concentration of substrates for colon bacteria is higher in the cecum of rodents and proximal colon than in the distal colon; therefore, the concentrations of SCFA are higher in these regions and decrease progressively toward the rectum. The majority of colon tumors occur in the distal colon. Therefore, if the production of SCFA is occurring predominantly in the proximal colon the putative protective effect of SCFA is not obtained. Pigs were fed diets supplemented with wheat bran, oat bran or baked beans. Pigs fed the bean diet, which contained greater amounts of RS, produced higher concentrations of SCFA in the colon. The bean diet produced greater amounts of butyrate and the fermentation occurred more distally in the colon (Topping et al 1993). Based on the fact that the protective effect of SCFA in the colon is related to the site of fermentation, Morita et al (1999) designed an experiment by adding psyllium and RS to

the diet. This study showed that the interactions between psyllium and RS made it possible to increase SCFA concentrations on the distal colon. Butyrate concentrations were specifically increased at this site. Another animal experiment by Govers et al (1999) showed that adding wheat bran to a diet with RS shifted the fermentation site of RS more distally in the colon. In general, the need for high levels of SCFA, especially of butyrate, at the distal colon may be important for protection against colorectal cancer. Thus, shifting fermentation of fiber and RS more distally through dietary manipulation could be an important step in this process.

C.1.3 Phytochemicals

There are many types of phytochemicals. Those found in beans include phenolic compounds, protease inhibitors, phytic acid and saponins (Craig, 1997). These compounds have gained interest among researchers because of their association with physiological and immunological effects. However more research is needed in this area to define roles and mechanisms of action.

C.1.3.1 Phenolic compounds

Phenolic compounds are one of the most representative phytochemical groups because they are the largest group and are widely distributed in plants. There is a great deal of interest in the area of phenolics because of the wide variety of phenolic compounds and because they are biologically active compounds that posses potential protective effects against many diseases. More than 8,000 structures have been identified, however quantification of these compounds in the diet is very difficult because their

concentration in foods vary depending on factors such as: maturity of product; temperature, light and water when grown; storage; and processing (King and Young 1999).

The phenolic compounds are classified into three main groups: flavonoids, phenolic acids and polyphenols or tannins (King and Young 1999). The flavonoids are the largest group and they are classified into the anthocyanins and the anthoxanthins. Compounds in the anthoxanthin group are the most studied, they have low molecular weights, and usually sugar molecules are bound to them. The anthocyanins are the molecules that give the red, blue and purple color pigment to many plants and foods, for example black beans (Takeoka, 1997). On the other hand, anthoxanthins are divided into flavonols, flavones, flavanols and isoflavones. These compounds are white-yellow.

Flavonols are one of the most important types of flavonoids in the diet. Quercetin, kaempferol and myricetin are the most common flavonols. These compounds are found in onions, apples and beans. Exposure of the product to U.V. light is essential for the production of flavonols and flavones. Therefore, the concentration of these compounds in the epidermis is much higher than inside. Storage can also alter the concentrations of certain flavonoids. Studies show that these compounds can be reduced during cooking and processing. The extent of the loss depends on the type of cooking and processing utilized.

Flavanols such as catechin and epicatechin are usually in combined forms with other compounds. The concentration of flavanols is higher in immature fruits and vegetables.

Isoflavones are found mainly in soybeans where they are in high concentrations (approx. 2000 mg/kg). Genestein and daidzein are the main types of isoflavones. These compounds are heat stable and very soluble in alcohol. Dry beans contain small quantities of isoflavones compared to soybeans.

The other group of phenolic compounds is the phenolic acids which include the hydroxybenzoic and hydroxycinnamic acids. The two main types of hydroxybenzoic acids are ellagic and gallic acid. On the other hand, caffeic and ferulic acid are the main hydroxycinnamic acids. These two components combine to form chlorogenic acid, which is heat sensitive and is found in high concentrations in fruits, vegetables and grains, especially in their epidermis.

The last group of phenolic compounds is the polyphenols or tannins. These compounds have high molecular weights and are grouped into hydrolysable and condensed tannins (Bravo et al 1995; Bravo, 1998). These compounds have the ability to bind to dietary proteins and minerals such as iron and to inactivate enzymes by binding with them. Condensed tannins are polymers of catechins and/or epicatechins that are present in legumes, fruits and grains. Tannins are found in greatest concentration in the epidermis of these foods. On the other hand, hydrolysable tannins are polymers of gallic and/or ellagic acids and are found mainly in berries, beans and nuts. The amounts of polyphenols in beans vary according to the outer seed color, therefore cooked black beans have the highest amounts (1.02mg%) of tannins and cooked white beans have the lowest amounts (0.28 mg%) (Bressani et al 1982; Bravo et al 1995; Bravo, 1998).

Several studies have demonstrated that the seed coat color of the dry bean is determined by the amount and the presence of flavonol glycosides, tannins and

anthocyannins (Beninger, et al 1998; Beninger, et al 1999; Beninger and Hosfield 1999). Beninger and Hosfield (1999) showed that red kidney beans contain three main types of flavonols: 3′,4′,5,7-tetrahydroxyflavonol-3-o-B-D-glucopyranosyl(2-1)o-B-D-xylo-pyranoside, quercetin-3-o-B-D-glucopyranoside and kaempferol-3-o-B-D- glucopyrano-side (Beninger and Hosfield 1999). However, the red color in these beans is not due to the flavonols but to the polymerized tannins present in these beans (Beninger, and Hosfield 1999). On the other hand the manteca beans, which are a yellow seed coated beans, have two types of flavonols that provide the seed color: (kaempferol (3,4,5,4′-tetrahydroxyflavone)-3-o-B-D)glucopyranoside and kaempferol-3-o-B-D-glucopyrano-side (Beninger, et al 1998; Beninger, et al 1999). The black or purple color observed in black seeded dry beans is due mainly to the high concentrations of three main anthocyanins: delphinidin 3-o-glucoside (56%), petunidin 3-o-glucoside (26%) and malvidin 3-o-glucoside (18%) (Takeoka, 1997). This study reported that anthocyanins in the black beans was approximately 213 ± 2mg per 100g of beans, which is very similar to the amounts found in blueberries.

C.1.3.2 Protease inhibitors

Proteases inhibitors are substances that are capable of inhibiting the activity of certain proteolitic enzymes. These compounds are found in many types of plants but especially in legumes. The main types of protease inhibitors in beans are the trypsin and chymotrypsin inhibitors (Weed et al 1985, Yavelow, et al 1983). Therefore these compounds could decrease the digestion of proteins. However, these compounds are heat

sensitive and when the food is cooked, much of the protease inhibitor activity is destroyed, which greatly improves protein digestibility.

C.1.3.3 Phytic acid

Phytic acid (inositol hexaphosphate) can be found in many foods of plant origin, especially cereals and legumes. Phytic acid is recognized as a chelating agent that affects the bioavailability of many minerals mainly copper, iron, manganese and zinc. Challa et al (1997) showed in their study that when phytic acid was 2% of the diet, weight gain by rats was reduced.

C.1.3.4 Saponins

Saponins are surface-active sterols or triterpene glycosides. They are present in a wide variety of plants such as legumes. These compounds are composed of pentoses and hexoses that are linked to a non-polar group known as sapogenin. Because of their amphiphilic characteristic, saponins have the ability to form stable foams in water (Oakenfull, 1981).

Several studies suggest that saponins can be toxic especially if these compounds enter the blood stream (Oakenfull, 1981). Coulson and Evans (1960) showed that saponins could depress the growth rate in rats (Coulson and Evans 1960). However other studies failed to support this conclusion.

Scientists have identified five different saponins in P. vulgaris. The sapogenin identified was soyasapogenol C and the different sugars attached were glucose, galactose, arabinose, rhamnose and glucuronic acid (Kinjo et al 1998).

C.2 Protection against colon cancer

Dry beans contain several components that may protect against colon cancer.

C.2.1 Dietary fiber

Several studies have shown that dietary fiber and RS protect against colon cancer through one or more mechanisms. One of the first mechanisms proposed was based on the fact that dietary fiber has the ability of increasing fecal bulk by absorption of water and by reducing transit time. This not only dilutes promoting agents, but also decreases the exposure of the epithelium to the promotor. Studies show that the fecal volume could be increased by consuming insoluble and soluble fibers, because both types of fibers have the ability to absorb water. However, soluble fiber is completely degraded by colonic bacteria and the increase in fecal bulk that occurs when soluble fiber is consumed is due to an increase in bacterial mass (Fuchs et al 1999).

Another proposed mechanism is through modification of bile acid metabolism. Epidemiological studies have demonstrated that populations with high risk for colon cancer have greater concentrations of secondary bile acids in their feces than populations with low risk. Actually, several animal studies have demonstrated that infusion of secondary bile acids into the colon increased cell proliferation and colon cancer risk. This process can be inhibited by fermentation of soluble fibers and RS. When these substrates are fermented in the colon the pH decreases, causing inhibition of the enzyme which is responsible of transforming bile acids (Nagengast et al 1995; Lupton and Turner 1999; Earnest et al 1999, Fuchs et al 1999).

The third mechanism by which dietary fiber and RS could protect against colon cancer is through the production of SCFA, especially butyrate which has shown to have anti-neoplastic properties (Cummings et al 1987; Gamet et al 1992; McIntyre et al 1993; Van Munster et al 1994; Scheppach et al 1995; Heerdt et al 1997; Archer, et al 1998; Hylla et al 1998; Cousin, 1999). In vitro studies show that butyrate is capable of regulating gene expression and cell growth (Gamet et al 1992). Butyrate has the ability of prolonging doubling time in cells, suppressing proliferation, and inducing differentiation in several cancer cell lines (Barnard and Warwich 1993; Archer, et al 1998). Data show that butyrate can inhibit the enzyme histone deacetylase in the nucleus, allowing hyperacetylation of histones. This step permits relaxation of the DNA structure and facilitates the access of DNA repair enzymes to the damaged area in the DNA. In vitro studies also show that butyrate can induce apoptosis in several cancer cell lines (Gamet et al 1992). Butyrate also has an important role in maintaining the integrity of the mucosa.

Another mechanism that shows how fiber and RS can protect against colon cancer is by inhibiting the production of ammonia. When the substrates for fermentation in the colon are proteins and/or amino acids, the main end products are SCFA and ammonia (Birkett et al 1996). In vitro studies demonstrate that ammonia is cytotoxic and can stimulate cell proliferation thereby increasing the risk for colon cancer. Increasing the amounts of carbohydrate available for fermentation promotes bacterial growth and their need of nitrogen. Thus bacteria are stimulated to utilize the ammonia, which reduces the concentration of ammonia in the colon (Birkett et al 1996).

Recently studies demonstrate that RS as well as soluble fibers are fermented in the colon. This suggests that RS provides the same benefits against colon cancer, having

an important role in decreasing the formation of secondary bile acids and ammonia and increasing the production of SCFA, especially butyrate (Lee et al 1989; Cassidy et al 1994; Van Munster et al 1994; Cummings et al 1996; Hylla et al 1998).

C.2.2 Folate:

Folate is an essential vitamin. Deficiency of folate is associated with anemia and neural tube defects. Recent research suggests an association between folate and colon carcinogenesis (Giovannucci et al 1998; Ma et al 1997; Martinez et al 1999; Backus, et al 2000). A prospective cohort study, known as the Nurses' Health study with 88,756 women participants, demonstrated that higher intakes of folate were associated with a lower risk of colon carcinogenesis (75% reduced in risk). However they reported that the positive effects were observed after 15 years or more (Giovannucci et al 1998). Mechanisms to explain this association have not been elucidated but scientists are investigating various hypotheses. Folate is essential for regeneration of methionine, is a methyl donor for DNA methylation and is important for production of purines and pyrimidines for DNA synthesis. Therefore, when folate is low in the diet, DNA methylation and DNA synthesis may be altered. Furthermore, in early stages of colon carcinogenesis, hypomethylation of DNA and alterations of DNA synthesis are observed which indicates that folate is associated with this process (Giovannucci et al 1998; Ma et al 1997; Martinez et al 1999; Backus, et al 2000).

Dry beans are a good source of folate, providing approximately 400 ug per 100 g of beans. The recommended daily allowance for folate is 400 ug.

C.2.3 Phytochemicals

From a traditional nutrition context, several phytochemicals are considered antinutritional. However, it is becoming apparent that some of these phytochemicals can in fact protect against colon cancer.

C.2.3.1 Phenolic compounds

Most phenolic compounds have antioxidant properties and many have anticarcinogenic properties. As mentioned previously, the phenolic compounds are classified into three main groups: flavonoids, phenolic acids and polyphenols or tannins. Flavonoids have been studied widely not only because there are many types of flavonoids but also because these compounds have different functions. For example, they can function as antioxidants, can associate with proteins that control cell cycle and can alter gene expression (Kitts 1994; Kawamori et al 1995; Bravo, 1998; Hollman and Katan 1999). However, a large cohort study from the Netherlands that included 120,850 subjects and had a follow up of 4 years showed no association between flavonoid intake and colon cancer mortality (Goldbohm, 1995). Nevertheless, it is important to mention that quantification of consumption of flavonoids is relatively ambiguous at this point (Hollman and Katan 1999). Also, it is likely that four years is an inadequate amount of time to detect any differences in cancer mortality.

Studies have shown that flavonols such as quercetin and kaempferol, and the isoflavone genistein, can induce apoptosis and decrease proliferation in different cancer cell lines (Mahmoud et al 2000). Flavanols such as catechin and epicatechin have a weaker anticarcinogenic effect. The anti-neoplastic effect of these compounds is caused

by transcriptional inhibition of cyclooxygenase 2 (COX-2), lipoxygenase (LOX) and protein kinase (Rao et al 1995; Mutoh et al 2000). COX and LOX catalyze the initial reactions in arachidonic acid metabolism and regulate production of prostaglandins, leukotrienes and thromboxanes. COX 2 is an enzyme that is up regulated during inflammation, since it's the key enzyme in the biosynthesis pathway that produces prostaglandins. Studies show that COX metabolites modulate cell proliferation, tumor growth and even immune response (Mutoh et al 2000). On the other hand LOX metabolites influence chemotoxic responses, some hormonal secretion and tumor cell adhesion (Kitts, 1994; Rao et al 1995).

Flavones, such as 2 phenyl-4H-1-benzopyran-4-one, are apoptotic inducers in HT 29 and Caco-2 colon cancer cell lines. Flavone compounds can also decrease cell proliferation and increase cell differentiation in colon cancer cells. These functions are associated with changes in the mRNA levels of several cell cycle and apoptotic related genes, including COX-2, p53, p21, cyclin E and B, and nuclear transcription factors of the bcl-2 family proteins (bcl-Xl, bak and bax) (Wenzel et al 2000). Studies show that inhibiting or suppressing the expression of COX-2 decreases the risk of colon carcinogenesis. Flavones can decrease mRNA of COX 2 and transcriptional factors of NF-kB. NF-kB expression has been associated with inhibition of apoptosis in colon cancer cells (Elder and Paraskeva 1998; Hao et al 1999; Mutoh et al 2000; Wenzel et al 2000).

Flavones can activate caspase3, which is associated with induction of apoptosis, arrest of cell cycle in G2-M phase and inhibition of cell cycle progress by altering expression of cell cycle genes such as p21. Increased expression of p21 is related to

terminal differentiation and inhibition of proliferation in vivo and in vitro, therefore tumors are associated with decrease expression of p21 as a result of loss of functional p53. However, it is not clear if p21 induces apoptosis or if it just arrests cell cycle and increases differentiation (Wenzel et al 2000).

Flavones are also important in decreasing the expression of cyclin B and E. A decrease in cyclin B and E expression is associated with cell cycle control. Another factor associated with cell cycle control is the expression of the bcl-2 family, which are critical regulators of cell death and are involved in chromosome translocation. Over expression of bcl-2 is very common in many types of cancers (Wang et al 2000).

Another group of flavonoids that may have anticarcinogenic activity are the phenolic acids. Caffeic acid and ferulic acid are hydrocynnamic acids that have been shown to protect the body against carcinogenesis by inhibiting N-nitroso compounds formation. Normally nitrite reacts with secondary and tertiary amines and amides in the stomach and produce nitrosamines and nitrosamides, which are cytotoxic. Caffeic acid and to a limited extent ferulic acid, reduce nitrite to nitric oxide or forms C-nitroso-compounds and prevents the formation of nitrosamines (Kroon et al 1997; Hirose et al 1999; Kuenzig et al 1999; Mahmoud et al 2000). Other studies show that hydroxybenzoic acids such as gallic acid and chlorogenic acid inhibit the nitrosation of proline and methylurea, providing protection against colon carcinogenesis.

There are many types of polyphenols or tannins that have been recognized as anticarcinogens. Tannins, especially the hydrolysable tannins, such as tannic acid, cuphiin D1 and pent-o-galloyl-B-D-glucose, have anticarcinogenic activity when incubated with cancer cell lines (HL 60). These compounds induce apoptosis in a dose

dependent way by triggering events associated with cell death such as the loss of mitochondrial transmembrane potential, release of cytochrome C into the cytosol and regulation of several caspases and COX-2 (Kaul and Khanduja 1998; Pan et al 1999; Nepka et al 1999; Ye et al 1999; Yang et al 2000; Wang et al 2000). Wang et al (2000) also suggested that the ability of tannins to induce apoptosis is associated with the inhibition of bcl-2 expression.

C.2.3.2 Protease inhibitors

Protease inhibitors are another type of phytochemical that has been associated with suppression of carcinogenesis in vivo and in vitro. Several studies suggest that these compounds have the ability to prevent over expression of oncogenes such as c-myc and c-fos. It is important to note that these compounds selectively inhibit over expression of these oncogenes without interfering with the normal expression of other genes (Billings et al 1991; St Clair and St Clair 1991; Kennedy, 1994). The main type of protease inhibitor present in the soybeans is the Bowman Birk inhibitor, which have shown to decrease carcinogenesis in several types of cancers such as colon cancer (Yavelow, et al 1983; Weed et al 1985). Dry beans also contain protease inhibitors, but much less is known about the inhibitors in dry beans compared to soybeans.

C.2.3.3 Phytic acid

Phytic acid has been identified as a compound that has anticarcinogenic properties as well as antioxidant properties (Graf and Eaton 1990). This compound is present in many fiber rich foods but especially in cereals and legumes. Several studies have

demonstrated that phytic acid is capable of reducing the risk of colon cancer. Challa et al (1997) demonstrated in their study that supplementation of the diet with phytic acid reduced significantly the incidence of aberrant crypt foci (Challa et al 1997). This compound is a natural antioxidant that has a very high binding affinity with minerals such as iron. By chelating iron, this compound suppresses iron catalyzed oxidative reactions and inhibits the oxidative damage to biological material (Graf and Eaton 1985; Graf and Eaton 1990; Graf and Eaton 1993; Takaba et al 1997).

C.2.3.4 Saponins

Saponins have been associated with anti-inflammatory and antioxidant properties, as well as cholesterol lowering effects. On the other hand several studies have shown that saponins and drugs containing saponins have antitumor activity against chemically induced carcinogenesis (Kawamori et al 1995; Konoshima, 1996; Koratkar and Rao 1997). Koratkar and Rao (1997) showed that adding 3% saponin to the diet was capable of reducing significantly the incidence of aberrant crypt foci. They suggest that this antitumor activity could be associated with antioxidant activity and specifically free radical scavenger function. Sidhu et al (1986) demonstrated in their in vitro study that the saponins have the ability to bind bile acids and cholesterol, therefore inhibiting the formation of secondary bile acids, which are cytotoxic to the colon.

C.2.4 Insulin

Recently, scientists have begun to associate insulin resistance and colon carcinogenesis. Mckeown-Eyssen and Giovannuci proposed a unifying hypothesis, which

may explain how Western type diets, obesity, physical inactivity and consumption of alcohol, increase colon carcinogenesis (McKeown-Eyssen 1994; Giovannucci, 1995). This proposed mechanism was based on the similarities of risk factors between colon cancer and non-insulin dependent diabetes mellitus. The hypothesis suggests that insulin resistance, more specifically hyperinsulinemia may stimulate growth of tumors (McKeown-Eyssen 1994; Giovannucci, 1995; Tran et al 1996; Kim, 1999; Liljeberg et al 1999). It has not been well documented that insulin stimulates growth of colon tumors, however insulin is an important growth factor for colon mucosa and exerts mitogenic effects in colon cancer cell lines in vitro. Colon cancer tissue has insulin and insulin like growth factors 1 (IGF-1) receptors and insulin may act as a growth factor through this receptor (Tran et al 1996; Will et al 1998; Kim, 1999).

Several epidemiological studies have failed to find an association between high levels of insulin and increased colon cancer risk. Nevertheless, these studies were small and had few subjects with colon cancer. Recently, a prospective study known as the Cancer Prevention Study of the American Cancer Society assessed risk factors for cancer and determined if the subjects with diabetes mellitus had a higher propensity of developing colon cancer. This study included 510,850 women and 352,849 men and after a follow up of 13 years, 4006 women and 3218 men developed colon cancer. This study demonstrated that men who had diabetes mellitus had a 30% increased risk of developing colon cancer and while women had a 16% increased risk when compared to subjects without diabetes mellitus (Will et al 1998).

On the other hand, a study in animals that were injected with insulin five times per week showed a positive effect between hyperinsulinemia and colon carcinogenesis.

Rats injected with insulin had a colon cancer incidence of 79% while the control rats had an incidence of 50% (Tran et al 1996). Taken together, these studies strongly suggest that there is an association between hyperinsulinemia and colon carcinogenesis. However, more studies are needed to prove the hypothesis and to determine the mechanism of action.

C.3 Other benefits of dry beans

Dry bean consumption has been associated with protection against a variety of chronic diseases. Many studies suggest an important protective effect against cardiovascular diseases, based on the potential of decreasing serum cholesterol concentrations. Furthermore, dry beans are a nutritious food that provides high amounts of protein, soluble and insoluble dietary fiber and vitamins and minerals and low amounts of fat. This type of food is recommended to prevent cardiovascular diseases (Anderson, 1986; Anderson, and Gustafson, 1988; Anderson, et al 1999).

Consumption of dry beans has been recommended for weight loss because they provide high amounts of fiber that increase satiety by increasing bulk (Anderson, 1986). Also, beans have a low glycemic index which helps to reduce the risk of developing diabetes mellitus and the tendency to develop obesity. Persons with diabetes mellitus also obtain benefits by consuming dry beans, because the presence of soluble and insoluble fiber reduces hyperglycemias and slows gastric emptying, contributing to the slow absorbance of glucose and consequential normalization of insulin levels (Kushi et al 1999).

III. RATIONALE

Dry bean is a major food legume in Latin America and many east and west African countries. Because of their low cost and availability, dry bean is an important source of protein and dietary fiber in these regions of the world. Limited epidemiological data shows an inverse association between colon cancer and consumption of dry beans. Correa (1981) showed a negative correlation of -0.68 between consumption of dry beans and incidence colon cancer in fifteen countries.

Hughes et al (1997) conducted the only animal study relating beans consumption to incidence of colon cancer. These authors demonstrated that eating pinto beans could reduce significantly colon carcinogenesis in rats. In this study the authors fed beans or casein diets for 34 weeks. The study showed that the rats fed the bean diet had fewer colon adenocarcinomas per rat, what indicates that beans have a protective effect.

The interest of studying dry beans and its effect on colon cancer, arises from the fact that beans contain several phytochemical components that when utilized as pure compounds have been linked to a reduction of incidence of colon cancer. Some of these components include protease inhibitors, phenolic compounds, saponins and phytic acid. Another characteristic of dry beans that has been associated with anticarcinogenic properties is the nature of starch and dietary fiber present in beans. The mixture of RS along with indigestible fiber may lead to an increase of SCFA in the distal colon where most tumors arise. In vitro data show that butyrate protects against colon cancer by regulating gene expression, cell differentiation and cell growth. On the other hand, dry beans have a low glycemic index that has been associated with protection against colon

carcinogenesis (Mckeown-Eyssen, 1994; Giovannuci 1995). They hypothesize that hyperinsulinemia and insulin resistance, stimulates growth of colon tumors.

The present study was designed to determine if consuming beans would reduce colon carcinogenesis. Two dry bean cultivars, representative of the navy and black bean market classes were chosen because of their marked differences in polyphenols (navy bean 0.28 mg% and black bean 1.02 mg%) and anthocyanins (black bean 213 mg% and navy bean 0 %) content (Leaky, 1995). Both of these cultivars are consumed in the US and Latin American countries. Therefore, the objective of this study was to determine if consumption of black beans or navy beans would reduce colon carcinogenesis in rats.

IV. OBJECTIVES

The main objective of this study was to determine if eating beans would reduce carcinogenesis in a rat model of human colon cancer.

V. NULL HYPOTHESES

The hypothesis to be tested is: Consumption of black and navy beans does not reduce the incidence and multiplicity of colon cancer in rats.

VI. APPROACH

It is known that dry beans have low protein digestibility and inadequate amounts of sulfur amino acids. For the first experiment it was necessary to ensure that rats fed bean diets were absorbing adequate quantities of essential amino acids so that protein intake would not affect growth. Therefore the first experiment determined protein digestibility of bean and casein protein so that diets could be formulated to provide equal amounts of absorbable essential amino acids for the second experiment.

In addition, it was necessary to determine if eating beans produces a high proliferation rate in colon epithelial cells compared to a control diets. If so, then feeding beans during carcinogen administration would be expected to produce more initiated cells which would likely lead to more colon tumors.

The second experiment was a colon cancer initiation-promotion study. Black beans and navy beans were selected to determine if anthocyanins and/or tannins were important in colon carcinogenesis. If either or both classes of phytochemicals are important to colon carcinogenesis then there should be a difference between navy and black beans.

In the second experiment the rats were fed the navy bean, black bean and control diets for four weeks before the injection of the carcinogen. Food consumption and growth of the rats were monitored through out the study. Later, starch in ileal contents and SCFA (butyrate) in distal contents were determined. Body composition was measured to determine if differences in body weight were due to percentage of fat and/or lean tissue. And tumor parameters were measured to see impact of dietary treatments.

VII. MATERIALS AND METHODS

Animals and housing:

Male Fischer rats (F-344) (21 days old) were obtained from Harlan Sprague-Dawley Indianapolis IN. They were housed three per plastic cage with sawdust bedding and were assigned to treatment groups by weight. The animal room was temperature (23-25°C) and humidity (40%-70%) controlled with a 12 hour off/on light cycle. The rats were given free access to the diet and to distilled water throughout the study.

Experiment 1 was designed to determine if beans altered cell proliferation rates in the colon. The second purpose was to determine the digestibility of protein from casein, black beans and navy beans. This study utilized 24 male Fischer rats (F-344) that were fed for 25 days.

The beans used in experiment 1 and 2 were grown in Michigan and obtained from Bayside Best Beans. The beans were soaked over night in distilled water at 4°C and cooked in open kettles until soft. They were then dried in a convection oven at 50-60°C. The dry beans were ground to pass through a screen with 1.6 mm diameter holes prior to mixing with other diet ingredients. Table 2 shows the composition of the diets for experiment 1.

Experiment 2 was designed to determine if feeding black or navy beans would alter colon carcinogenesis. The protein digestibility was 66% for black beans, 76% for navy beans and 95% for casein. In experiment 2 diets were formulated to provide 12.69g of digestible protein per 100g of diet (Table 3).

Table 2

Diet composition for experiment 1[a]

Ingredients	Bean diet	Control diet
Bean (black or navy)	64.8	-
Casein	-	19.0
Cystine	-	0.3
Sucrose	24.4	57.9
Fiber (cellulose)	-	11.0
Soybean Oil	6.0	7
Minerals[b]	3.5	3.5
Vitamins[b]	1	1
Choline bitartrate	0.25	0.25
BHT[c]	0.0014	0.0014
TOTAL	100	100

[a] Values are presented per 100 g of diet. All diets contained 17.5% protein, 11% fiber and 7% fat.

[b] Vitamin mix AIN 93G VX and mineral mix AIN 93G MX (Reeves et al, 1993). The mineral mix did not contained calcium.

[c] BHT= Butylated hydroxytoluene.

Table 3

Diet composition for experiment 2 [a]

Ingredients	Black bean	Navy bean	Control
Beans	75.0	75.0	-
Casein	2.6	-	14.5
Cysteine	0.138	0.121	0.218
Methionine	0.100	0.121	-
Corn oil	-	-	1.1
Tallow	11.2	11.2	11.2
Soybean Oil	3.6	3.6	3.6
Cornstarch	2.3	4.9	51.5
Fiber (cellulose)	-	-	12.7
Calcium carbonate	0.25	0.25	0.25
Minerals [b]	3.5	3.5	3.5
Vitamins [b]	1.0	1.0	1.0
Choline bitartrate	0.25	0.25	0.25
BHT [c]	0.0032	0.0032	0.0032
TOTAL	100.00	100.00	100.00

[a] Values are presented per 100 g of diet. The total protein content for the black beans was 21.1 g of protein/100 g of beans, for navy beans was 22.26 g of protein/100 g of beans and casein was 92 g in100 g. All diets contained 12.69% digestible protein, 16% fat and 12.75 % fiber.

[b] Vitamin mix AIN 93G VX and mineral mix AIN 93G MX (Reeves et al, 1993). The mineral mix did not contained calcium.

[c] BHT= Butylated hydroxytoluene

All diets contained the same amount of total fat (16%). The composition of fat in the beans is similar to corn oil, so an equal amount of corn oil was added to the control diet. Total fat and the ratios of saturated, polyunsaturated and monounsaturated fatty acids were based on the typical American diet (Ernst, 1997). It is well known that dry beans are low in sulfur amino acids, so the bean diets were supplemented with cysteine and methionine to meet the amino acid needs of growing rats (Reeves, 1993) Cysteine was added to the control diet to make casein a complete protein. In experiment 2 the rats were fed the diets for a total of 36 weeks and they were weighed every two weeks.

Administration of carcinogen:

After one month of feeding the diets, the colon carcinogen azoxymethane was injected (15mg/kg of body weight) subcutaneously in the flank. Two injections were administered with one week between injections.

Food consumption and protein digestibility:

To measure food consumption groups of three rats were housed in stainless steel hanging wire cages to collect spilled food and feces. Food consumption was calculated as initial weight of food jar minus weight of food jar at the end minus the weight of spilled diet. The measurement was for a six-day period and results are presented as the average food intake per rat.

In experiment 2 eighteen rats from each treatment were used to determine food consumption every two months throughout study.

In experiment 1 in order to obtain the protein digestibility it was necessary to determine the exact amounts not only of food consumed but also to collect the feces. The feces were dried, ground and a 0.300 g sample was transferred into a Kjeldahl digestion tube. Then a kjeltab, 1 ml of hydrogen peroxide (30%) and 6 ml of concentrated sulfuric acid were added to each tube and the tubes were gradually heated to 400°C until the liquid was colorless.

The digested samples were distilled in a Buchi 323 distillation unit. Sodium hydroxide (30%), water and steam were added. The nitrogen was recovered in the distillate and collected in a flask containing boric acid (4%). Then the samples were titrated with HCL (0.0865 N) to determine the amount of nitrogen present. Protein was calculated as N (6.25).

% apparent protein digestibility was calculated as: $\frac{\text{protein intake} - \text{protein excretion}}{\text{protein intake}} * 100$

Necropsy:

The rats were sacrificed using CO_2 inhalation and exsanguination. The entire abdominal cavity was examined visually for tumors. Then the colon was removed and cut open longitudinally and rinsed with warm tap water. The entire colon was pinned to cardboard and fixed in 4% buffered formaldehyde (pH 7.4). Then all suspected tumors were excised, weighed and processed using routine histologic procedures. A 2 cm section approximately 4 cm proximal to the anus was similarly processed. All suspected tumors were sectioned, stained with hematoxylin and eosin and classified by a pathologist. Adenomas and adenocarcinomas are reported as tumors.

Cell proliferation was determined in experiment 1. Two cm of the distal colon, approximately 4 cm from the anus, were collected and processed as above. This tissue was stained and the crypt height, labeling index, proliferation zone and labelling index of every third of the crypt was determined. Proliferating cells were identified by immunohistochemistry utilizing the primary antibody to proliferating cell nuclear antigen (PCNA). This method was described by Miyagi et al (2000).

In experiment 2 the ileal and distal colon contents were collected from 15 rats per treatment. The collection of these contents was done immediately after the excision of the ileum and the colon. The contents were weighed and stored at -20°C for analysis of SCFA and resistant starch.

Body composition:

Fat was extracted from 15 randomly selected rats per treatment. The carcasses were digested with ethanolic sodium hydroxide (35% 3M NaOH - 65% ethanol). The carcasses were heated at approximately 70°C until all soft tissue was dissolved. When the carcasses were completely solubilized, the mixture was placed into a separatory funnel and acidified with concentrated HCL. Hexane was added, the funnel was shaken and the mixture was allowed to separate into hexane and aqueous layers. The hexane extraction was repeated 3 times and the 3-hexane extractions were collected into another separatory funnel to facilitate further separation of the hexane and water phases. The hexane layer was filtrated through anhydrous sodium sulfate to eliminate traces of salt and water. The hexane-fat mixture was collected in a tared round bottom flask. The hexane was

evaporated and weight of the fat determined. Lean tissue was calculated by subtracting body fat from the carcass weight.

Resistant starch analysis:

Ileum contents were weighed and 2N NaOH was added in an amount equal to 3X the weight of the sample. The NaOH was added to dissolve the starch in the sample. After one hour the mixture was centrifuged for 15 minutes and the supernatant was collected and weighed. An equal weight of 2N HCL was added to neutralize the sample. Then the pH was adjusted to 4.5 by adding a 1M NaAc - 0.1M $CaCl_2$ buffer in an amount equal to two times the volume of the HCl added previously. The mixture (490 ul) was combined with 10 ul (3 enzyme units) of amyloglucosidase (from aspergilius niger). The test tubes were capped and heated at 60°C over night to complete starch digestion.

The next day, 2.5-10ul of the amyloglucosidase digest was placed in a test tube and double distilled water was added to make the total volume 100ul. Then 1ml of the combined color-enzyme solution was added and they were incubated at room temperature for 45 minutes. The reagent kits (# 510A) were purchased from Sigma St Louis, MO. and the supplier's directions were followed. All samples were kept in the dark during color development. Absorbance was measured at 450 nm. Standards were prepared using 0, 4, 8, 12, 16 or 20ug of glucose per cuvette (1.1ml/cuvette). The glucose content of each sample was calculated using a linear equation obtained from the standards and adjusting for dilution factors. Starch was calculated as 0.9 (glucose). The starch analyses were done in duplicates. Amyoglucosidase, PGO enzymes and o-dianisidine dihydrochloride were obtained from Sigma.

SCFA analysis:

To measure the SCFA in the distal colon contents, the samples were mixed with 2 volumes of double distilled water and one volume of 25% wt/wt meta-phosphoric acid in a 1:1:2 ratio (ml meta-phosphoric acid:sample wt:ml water). The samples were centrifuged at 13,000 x g for 15 minutes and the supernatant was used to measure SCFA by gas chromatography and flame ionization detection. The SCFA were separated on a 1m long x 3mm ID glass column packed with 15% SP-1220, 1% H_3PO_4 on 100/120 Chromosorb W AW. Nitrogen was the carrier gas. Initial column temperature was held at 80°C for 3 min and then the temperature was increased 3°C per minute to the final temperature of 98°C. Acetate, propionate, isobutyrate, butyrate, valerate and isovalerate in the samples were determined by the external standard technique. Duplicates of each sample were run until duplicates varied less than 5%. Concentrations were calculated taking into account the dilution factor and the weight of colon contents.

Statistical analysis:

The statistical software SPSS was used for the statistical analysis of all data. One-way analysis of variance (ANOVA) was used to compare means for final body weight, food consumption, SCFA, resistant starch, body fat and tumor multiplicity. Post hoc analysis was done by the Fisher's LSD multiple comparison test to identify treatment differences. The Chi square test was used to assess differences in tumor incidence. Since the variance for tumor weight per rat was non-homogeneous the tumor burden was

analyzed by the Wilcoxon Rank Sum procedure. Data is reported as mean ± standard error of the mean unless otherwise stated. An alpha value of 0.05 was used to determine statistical significance.

VIII. RESULTS

Cell proliferation:

There were no significant differences in crypt height, labeling index, proliferation zone or labelling index in the bottom, middle or upper third of the crypt among treatments. This indicates that consumption of black beans or navy beans did not increase cell proliferation in the colon mucosa as compared to controls (Table 4) .

Protein digestibility:

The protein digestibility for black beans was 66%, for navy beans was 76% and for casein was 95%.

Growth and food consumption:

There was a significant difference in final weights among treatments. Rats consuming the two market classes of beans diets weighed significantly less than the control group. Table 5 summarizes the differences between the treatments and Figure 1 shows the evolution of weight gain per treatment through out the study. The graph shows that the difference in weight was due to a gradual increase between animals fed beans and the control diet.

There were significant differences in food consumption. Rats consuming the navy bean diet ate the least and the rats consuming the control diet ate the most (Table 6). The rats consuming the black bean diet also ate significantly less than the rats in the control diet.

Table 4

Proliferating Cell Nuclear Antigen in colon crypts of rats fed black beans, navy beans or control diets.*

Diets	Crypt Height	Labelling Index	Proliferative Zone	Labelling Index Bottom 1/3	Labelling Index Middle 1/3	Labelling Index top 1/3
Black bean	33.88 ± 2.95[a]	0.39 ± 0.07[a]	0.64 ± 0.07[a]	0.53 ± 0.17[a]	0.54 ± 0.13[a]	0.09 ± 0.05[a]
Navy bean	33.43 ± 1.94[a]	0.40 ± 0.08[a]	0.65 ± 0.07[a]	0.56 ± 0.14[a]	0.57 ± 0.15[a]	0.07 ±0.04[a]
Control	33.94 ± 3.68[a]	0.41 ± 0.05[a]	0.66 ± 0.04[a]	0.56 ± 0.14[a]	0.60 ± 0.13[a]	0.08 ± 0.03[a]

*Values are presented, as mean ± SEM. Means in a column with different superscripts are significantly different at a P-value < 0.05.

Table 5

Final weight and weight gain for rats fed black beans, navy beans or control diets.*

Diets	N	Final weights (g)	Weight gain (g)
Black beans	32	379 ± 25.2 [a]	338 ± 23.4 [a]
Navy beans	28	375 ± 20.9 [a]	334 ± 20.5 [a]
Control	28	428 ± 26.5 [b]	387 ± 25.0 [b]

*N=number of rats per treatment. Values are presented, as mean ± SD. Means in a column with different superscripts are significantly different. P-value < 0.05

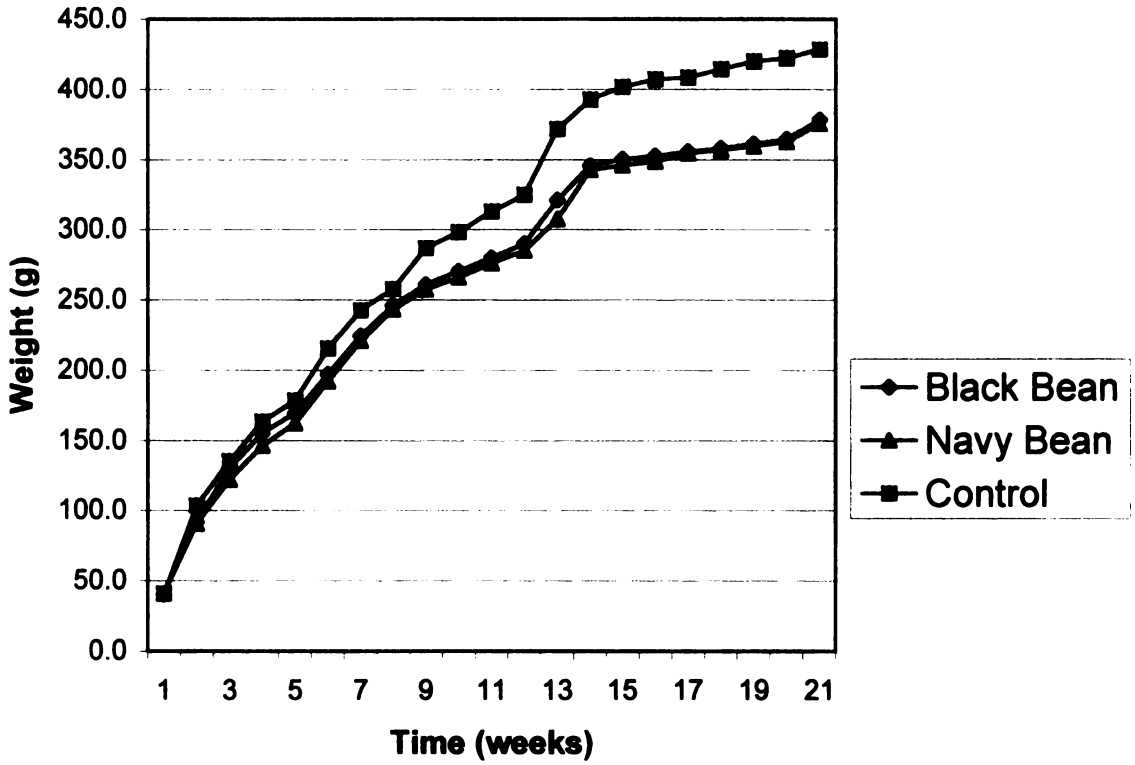

Figure 1
Changes in weight of rats fed black beans, navy beans or control diets.

Table 6

Average food consumption for rats fed black beans, navy beans or control diets.*

Diets	N	Food consumption (g/day/rat)
Black beans	18	11.9 ± 0.3^{b}
Navy beans	18	10.4 ± 0.2^{a}
Control	18	13.0 ± 0.4^{c}

*N=number of measurements. Values are presented, as mean ± SEM. Means in a column with different superscripts are significantly different. P-value < 0.05

Body composition:

Body composition was significantly affected by diet. There was a significant difference in the percentage of body fat and lean tissue among all treatments. The rats fed navy beans had the lowest percentage of body fat and rats consuming the black bean diet had an intermediate percentage of body fat while the rats fed the control diet had the highest percentage body fat. Lean body mass followed the same pattern as percentage body fat, i.e. navy bean < black bean < control. The lean body mass for the rats fed beans was 90% and 93% of the lean body mass of control rats. Table 7 summarizes the results for body composition.

Resistant starch:

Rats eating the beans diets had significantly more starch in their ileal contents than rats fed the control diet (Table 8). There was no significant difference in the amount of resistant starch in ileal contents from the two beans diets. This shows that there is significantly more starch that escapes digestion in the small intestine when beans are consumed.

SCFA analysis:

The concentrations of SCFA in contents of the distal colon are shown in Table 9. There was a significant treatment effect in the concentration of several SCFAs. The concentration of acetate in the colonic contents of rats consuming the beans diets was significantly higher than for the rats consuming the control diet, however the concentration was the greatest for rats eating the navy bean diet. On the other hand the

Table 7

Body fat and lean body mass for rats fed black beans, navy beans or control diets.*

Diets	N	Body fat (g)	Lean body mass (g)
Black beans	15	73.7 ± 2.3^{b}	299.3 ± 4.2^{b}
Navy beans	15	63.7 ± 2.8^{a}	295.7 ± 3.6^{a}
Control	15	104.2 ± 3.8^{c}	325.1 ± 4.1^{c}

* N=number of samples used per treatment. Values are presented, as mean ± SEM. Means in a column with different superscripts are significantly different. P-value < 0.05

Table 8

Resistant starch in ileal contents of rats fed black beans, navy beans or control diets.*

Diets	N	Resistant starch (mg/g of dry wt)
Black beans	15	1165 ± 131^{b}
Navy beans	15	1103 ± 128^{b}
Control	15	69 ± 3^{a}

* N=number of rats used per treatment Values are presented, as mean ± SEM.

Means in a column with different superscripts are significantly different. P-value < 0.05

Table 9

Molar proportions of SCFA in colon contents of rats fed black beans, navy beans or control diets.*

Diets	Acetate	Propionate	IsoButyrate	Butyrate	Isovalerate	Valerate
Black beans	32 ± 2.8 [b]	3.0 ± 0.2 [a]	T	18 ± 2.1 [b]	T-0.8	T-1.2
Navy beans	47 ± 5.3 [c]	4.1 ± 0.5 [b]	T- 0.7	18 ± 3.4 [b]	T-1.5	T-2.3
Control	7 ± 0.7 [a]	3.5 ± 0.2 [ab]	T- 1.0	2 ± 0.2 [a]	T-1.9	T-1.8

*Values are presented as mean ± SEM. Means in a column with different superscripts are significantly different. P-value < 0.05. For isobutyrate, isovalerate and valerate the values are presented as a range from T (trace) to the highest amount.

differences for propionate were minor even though it reached statistical significance. The concentrations of butyrate were significantly higher in the colon contents from rats fed the two beans diets compared to rats fed the control diet. It is important to emphasize the high concentration of butyrate at the distal colon in the rats consuming the beans diets. The concentrations of isobutyrate, isovalerate and valerate were very small and therefore results are expressed as a range.

Tumor distribution:

Tumor data are shown in Table 10. Colonic tumor incidence (% of animals that had one or more tumors) was significantly lower in rats fed the diets containing beans. Likewise, colon tumor multiplicity (number of tumors/tumor bearing rat) was significantly lower in rats fed the beans diets. There was no difference in tumor incidence or tumor multiplicity between rats fed diets containing black beans or navy beans. Small intestine tumor incidence and multiplicity followed the same general pattern as for the colon data. However, tumor burden (g tumor/tumor bearing rat) were not significantly different among treatments (Table 11).

Table 10

Colon tumor incidence and multiplicity and small intestinal tumor incidence of rats fed black beans, navy beans or control diets.*

Diets	N	Colon tumor incidence	Colon tumor multiplicity	Small intestine tumors incidence
Black beans	32	28% [a]	1.1 ± 0.33 [a]	3% [a]
Navy beans	28	25% [a]	1.0 ± 0 [a]	18% [a]
Control	28	61% [b]	2.2 ± 1.18 [b]	46% [b]

* N=number of rats per treatment Values are presented as mean ± SEM. Means in a column with different superscripts are significantly different at a P-value < 0.05.

Table 11

Colon tumor burden for rats fed black beans, navy beans or control diets.*

Diets	N	Colon tumor weight (g)
Black beans	9/32	0.08 ± 0.12^{a}
Navy beans	7/28	0.07 ± 0.07^{a}
Control	17/28	0.16 ± 0.30^{a}

*N=number of rats with colon tumors/ effective number of rats per treatment. Values are presented as mean ± SEM. Means in a column with different superscripts are significantly different. P-value < 0.05

IX. DISCUSSION

The main objective of this study was to determine if consumption of black beans and/or navy beans would reduce colon carcinogenesis in rats. The data in Table 9 shows that eating black or navy beans had a profound impact on AOM induced colon carcinogenesis. Eating black beans decreased tumor incidence by 54% and eating navy beans reduced tumor incidence by 59%. Similarly, tumor multiplicity was decreased by 50% and 55% by feeding black and navy beans respectively.

The only study that has examined the effect of feeding dry beans and colon carcinogenesis was done by Hughes et al (1997). They demonstrated that consumption of pinto beans inhibited chemically induced colon carcinogenesis. They fed pinto beans or casein diets to rats for 34 weeks. The pinto bean diet significantly lowered the incidence of colon tumors (24%) compared to the control diet (50%). In this study we found similar results to Hughes et al.

It is well known that energy restriction reduces colon cancer. In the present study the rats fed the navy bean and the black bean diets ate less and grew slower than the control group. All three diets were equivalent for digestible protein, minerals, vitamins, fat, fiber and energy content. All essential amino acids were present in sufficient amounts to allow normal growth. The differences in growth were directly associated with food consumption. Table 6 shows that the rats eating black beans ate 8.5% less food and rats eating navy bean diet ate 20% less food. These differences in food consumption may have been due to the low glycemic index of beans and/or the presence of certain phytochemicals in beans that could depress appetite. Beans are slowly digested producing

low glycemias, controlled insulin responses and increased satiety (Leeds, 1981; Leeds, 1982). Given that a 30-40% food restriction is usually required to significantly reduce tumorgenesis, it is unlikely that the 8.5-20% reduction in food intake was the major factor for the very strong reduction in colon cancer that was observed.

The reduction in colon cancer by feeding beans may be related to a low glycemic index in a second way. Foods that produce a low glycemic index, such as dry beans, produce lower plasma glucose levels, which tend to lower the secretion of insulin (Leeds, 1981; Leeds, 1982; Liljeberg et al 1999). It is proposed that insulin levels promote colon carcinogenesis (Mckeown-Eyssen, 1994; Giovannuci, 1995). This hypothesis suggests that the hyperinsulinemia and insulin resistance, usually observed in people with non-insulin dependent diabetes mellitus and/or overweight, stimulates growth of colon tumors. Trans et al. (1996) confirmed the hypothesis in their study when they injected insulin into rats, and showed an increased colon tumor incidence of 79% compared to 50% in the control group (Trans et al 1996).

The low glycemic index associated with beans may be related to the results in yet a third way. A low glycemic index has been shown to protect against obesity. Both groups of rats consuming beans had significantly less body fat than the control group (Table 7). Lean body mass was less in the rats fed bean diets also. However, when the small amounts of protein associated with adipose tissue is considered, the bean fed rats had only 6-8% less non-adipose tissue. A positive energy balance in adults leading to excess weight and obesity has been suggested to increase the risk of colon cancer (Mckeown-Eyssen, 1994; Giovannuci, 1995).

RS was measured in the ileal contents to estimate the amount of starch that was entering the colon. Table 8 shows that the rats fed the bean diets had significantly higher amounts of RS entering the colon than the control group. The amount of acetate and butyrate in the distal colon contents was much higher for rats fed the bean diets. Starch is readily fermentable by colonic bacteria and starch fermentation selectively leads to increased butyrate compared to acetate and propionate. The interpretation of high levels of butyrate in the distal colon is that RS reached the distal colon and lead to greater fermentation, confirming that dietary substrates can influence which SCFA will be produced in greater amount (Ehle et al 1982). In vitro studies have shown that butyrate has anticarcinogenic effects by regulating gene expression and cell growth. Butyrate has the ability to suppress proliferation and induce differentiation, apoptosis and DNA repair (Gamet et al 1992; Hague et al 1993; Barnard and Warwich 1993). Therefore, the high levels of butyrate obtained in the distal content of the rats eating the beans diets suggest another possible mechanism through which dry beans reduced colon carcinogenesis.

It is known that tumors develop mainly in the distal colon, however the fermentation of RS and soluble fiber occur more proximal in the colon making the concentrations of SCFA high in the proximal colon and progressively lower towards the distal colon. Thus, the potential protection that could be derived from SCFA does not occur because starch does not reach the distal colon to produce butyrate where most of the tumors develop. Morita et al. (1999) manipulated the site of fermentation by mixing RS with physllium which resulted in a shift of fermentation towards the distal colon. The mixture of RS and fiber allowed the fermentation to occur more slowly throughout the colon and increased the concentration of SCFA more distally (Morita, 1999). Dry beans

have the characteristic of containing high amounts of RS as well as high amounts of soluble and insoluble fibers, therefore the fermentation of RS and soluble fiber is shifted more distally to where the production of butyrate would be more beneficial. This is confirmed by the high concentrations of butyrate observed in the distal content of the rats fed the beans diets.

Beans contain several classes of phytochemicals that have been associated with anticancer activity. Beans contain phenolic compounds, protease inhibitors, phytic acid and saponins. These phytochemicals may be partially responsible for the decrease in colon cancer incidence, however no data is provided to determine if they are important. Nevertheless, the differences in amounts of anthocyanins and tannins between beans show that the reduction in colon cancer incidence probably was not be due to these phytochemicals.

X. CONCLUSIONS

In this type of research when a whole food (as opposed to chemicals) is used, it is not possible to identify specific components of beans that are responsible for the observed reduction in colon cancer. It is likely that various phytochemicals have synergistic as well as antagonistic activities toward cancer. The most relevant observation is that eating black beans or navy beans significantly lowered colon tumor incidence and multiplicity.

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