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Whole Grain Consumption and Health of the Lower Gastrointestinal Tract: A Focus on Insoluble-Bound Phenolic Compounds

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1. Introduction

A whole grain is the intact, ground, cracked or flaked caryopsis, whose principal anatomical components - the endosperm, germ and bran - are present in the same relative proportions as they exist in the intact caryopsis (AACC, 1999). The endosperm, the largest component of the caryopsis contains starchy carbohydrates, proteins, vitamins and minerals and provides energy for the rest of the plant. The bran, the multi-layered outer skin of the grain, protects the germ and the endosperm from damage from sunlight, pests, water, and diseases. The germ or embryo is the part of the grain that becomes a new plant when fertilized by pollen.

Whole grain food products can be defined by one of two definitions. An intact whole grain food product is a product that has the original composition of bran, germ, and endosperm throughout the entire lifetime of the product, from field to consumption. A reconstituted whole grain food product is a product that has the original components of a whole grain recombined to the relative proportion naturally occurring in the grain kernel. Due to advances in food processing and the commonplace nature in which these processes take place, the bulk of the whole grain food products would be considered reconstituted whole grain products.

Many grains are consumed on a daily basis in a number of products from around the world. Wheat, *Triticum aestivum*, has become the prominent grain based on total consumption. Corn, *Zea mays* L., is another commonly eaten grain and is consumed as tortilla, popcorn, or corn cakes. Rice, *Oryza sativa* L., is the major staple for a majority of the world's population, especially in Asian countries. Rice is rarely eaten as a whole grain. Generally, the endosperm fraction, i.e. the polished rice, without the bran and germ fractions, is eaten. Rice can also be parboiled, incorporating B vitamins into the endosperm of the grain following heating of the whole grain. White rice is not considered a whole grain. Oats, *Avena sativa* L., are almost always eaten whole since their bran and germ fractions are rarely removed. They also tend to have a sweet taste, making good breakfast cereals and beers. Millet, *Panicum miliaceum* L., is rarely consumed by humans in North America, but is very common in Asia and also in Africa. Sorghum, *Sorghum bicolor*, is also rarely consumed in North America. Barley, *Hordium vulgare* L., has a tough hull that is difficult to remove and therefore requires long cooking times. Rye, *Secale cereale* L., has high fiber content in its endosperm and is consumed with

highest frequency in parts of Scandinavia and Russia. Pseudocereals are plants with seeds that can be milled and used much the same way as cereal flours (Brady *et al.*, 2007). These pseudocereals, in addition to rice, millet and maize, do not contain the protein gluten, intolerance to which is known as Celiac disease. Pseudocereals include buckwheat, quinoa, and amaranth. A list of common grains and grain-based food products is provided below (Table 1).

Species	Common Name	Common Food Products
<i>Zea mays</i>	Corn, Maize	Corn cakes, tortilla, popcorn, hominy
<i>Orize sativa</i>	Rice	White rice, brown rice, parboiled rice
<i>Triticum aestivum</i>	Wheat	Breads, flours, pasta, baked goods
<i>Secale cereal</i>	Rye	Breads
<i>Avena sativa</i>	Oats	Oatmeal, flour
<i>Triticum aestivum spelta</i>	Spelt	Breads, baked goods
<i>Hordom vulgare</i>	Barley	Hulled barley
<i>Sorghum bicolor</i>	Sorghum	Couscous, porridge, molasses
<i>Panicum miliaceum</i>	Millet	Porridge, millet
<i>Chenopodium quinoa</i>	Quinoa	Cooked quinoa, pasta,
<i>Amaranthus caudatus</i>	Amaranth	Breads, pasta
<i>Fagopyrum esculentum</i>	Buckwheat	Porridge, pasta, pancakes, breads

Table 1. Common grains and grain-based food products

2. The health benefits of whole grain consumption

Whole grain consumption has been associated with reduced risk of chronic diseases including cardiovascular disease, type 2 diabetes, obesity and some cancers (Okarter & Liu, 2010). The results from select epidemiological studies that investigated the association between increased whole grain consumption and reduced risk of chronic diseases are reported below.

2.1 Cardiovascular disease

Jacobs *et al* reported results from the Iowa Women’s Health Study. There was an inverse association between whole grain intake and risk of death from ischemic heart disease (IHD), after adjustment for potentially confounding factors and dietary fiber intake (RR = 0.70) (Jacobs *et al.*, 1998). After adjustment for total dietary fiber intake, Jacobs *et al* found there was still an inverse association between whole grain intake and IHD across all intakes of whole grain, RR = 0.77; 95% CI 0.54 – 1.10 for highest quintile of whole grain intake, highlighting that other factors in addition to dietary fiber may have contributed to the beneficial health effects.

Liu *et al* reported results from the Nurse’s Health Study (NHS). There was an inverse association between whole grain intake and risk of coronary heart disease (CHD) (RR = 0.51; 95% CI 0.41 – 0.64), for women in the highest quintile for whole grain consumption when compared to the lowest (Liu *et al.*, 1999).

Lockheart *et al* investigated the association between dietary patterns and risk of first myocardial infarction using the data from a case-control study performed in Norway. After adjusting for family history of heart disease, smoking, energy intake, and other possible confounding factors, consumption of whole grain breakfast cereals was inversely associated with risk of first myocardial infarction (RR = 0.64; 95% CI = 0.45 – 0.90) (Lockheart *et al.*, 2007) when comparing the group with the highest level of whole grain breakfast cereal intake to the group with the lowest.

Flint *et al* investigated the association between whole grain consumption and hypertension in men using data from the Health Professionals Follow-Up Study. After adjusting for fruit and vegetable consumption, smoking, family history of hypertension, physical activity and other possible confounding factors, whole grain consumption was associated with reduced incident of hypertension, when comparing the highest quintile of whole grain consumption to the lowest (RR = 0.81, 95% CI = 0.75-0.87) (Flint *et al.*, 2009).

Wang *et al* investigated the association between whole grain consumption and hypertension using data from the US Health Professional's Follow-Up Study. Whole grain consumption was inversely associated with hypertension when comparing the highest quintile of whole grain intake to the lowest, after adjusting for possible confounding lifestyle, clinical, and dietary factors (RR = 0.89; 95% CI = 0.82 – 0.97) (Wang *et al.*, 2007). Contrarily, no significant association was seen between refined grain consumption and hypertension.

The positive effects of whole grain consumption on cardiovascular parameters are thought to be mediated by improvements in body weight, dyslipidemia, and insulin resistance (Harris & Kris-Etherton, 2010). However, the Dietary Guidelines Advisory Committee 2010 rated the evidence for the protective relationship between whole grains and cardiovascular disease as “moderate” (Harris & Kris-Etherton, 2010). It is important to understand that causality cannot be assigned in observational studies such as the ones mentioned above. It therefore remains unknown whether or not whole grains are protective against cardiovascular disease. Whole grain consumption may be a marker of a healthy lifestyle (Harris & Kris-Etherton, 2010). It is also becoming more difficult to determine the amount of whole grains consumed due to the increasing number of new food products containing varying amounts of whole grain.

2.2 Type 2 diabetes and obesity

Obesity has been linked to the development of type 2 diabetes and cardiovascular diseases. Increased whole grain consumption has been associated with reduced risk of type 2 diabetes and obesity.

Meyer *et al* investigated the association between whole grain consumption and the relative risk of type 2 diabetes. Whole grain consumption was inversely associated with risk of type 2 diabetes when comparing the highest quintile of whole grain intake to the lowest (RR = 0.79; 95% CI = 0.65 – 0.96) (Meyer *et al.*, 2000).

Fung *et al* investigated the association between whole grain consumption and risk of type 2 diabetes, using the data from the US Health Professionals Follow-Up Study. After adjusting for confounding factors including fruit and vegetable consumption, whole grain consumption was inversely associated with risk of type 2 diabetes when the highest quintile of whole grain intake was compared to the lowest (RR = 0.70; 95% CI = 0.57 – 0.85) (Fung *et al.*, 2002).

Montonen *et al* investigated the association between whole grain intake and risk of type II diabetes using data from the Finnish Mobile Clinic Health Examination Survey (Montonen *et al.*, 2003). Whole grain consumption was inversely associated with type 2 diabetes when comparing the highest quartile of whole grain consumption to the lowest, after adjusting for fruit, berry, and vegetable consumption and other confounding factors, (RR = 0.65; 95% CI = 0.36 – 1.18).

Bazzano *et al* investigated the association between consumption of whole breakfast cereals and weight gain in men using data from the Physician's Health Study. After adjusting for baseline BMI, physical activity, age, and other possible confounding factors, whole grain breakfast cereal consumption was inversely associated with risk of having a BMI greater than 25 (RR = 0.83; 95% CI = 0.71 – 0.98) and body weight gain of more than 10 kg (RR = 0.78; 95% CI = 0.64 – 0.96), 8 years after initial subject evaluation (Bazzano *et al.*, 2005).

Newby *et al* investigated the association between whole grain consumption and BMI, weight, and waist circumference using data from the Baltimore Longitudinal Study on Aging. Consumption of whole grain was inversely associated with BMI, weight, and waist circumference when the highest quintile of whole grain consumption was compared to the lowest after adjusting for refined grain intake, total energy intake, and other possible confounding factors (Newby *et al.*, 2007).

Munter *et al* investigated the association between whole grain, bran, and germ intake using data from the first and second trials of the Nurse's Health Study. Whole grain intake was inversely associated with risk of type 2 diabetes in the first trial of the Nurse's Health Study (RR = 0.63; 95% CI = 0.57 – 0.69) and in the second trial of the Nurse's Health Study (RR = 0.68; 95% CI = 0.57 – 0.86) after adjusting for physical activity, total energy intake, and other possible confounding factors (Munter *et al.*, 2007).

The positive association between increased whole grain consumption and reduced risk of type 2 diabetes, obesity, and major weight gain is most likely due to the presence of complex carbohydrate and the slower release of sugars into the blood. For this reason, the inclusion of whole grain has been recommended as a preventative measure from type 2 diabetes.

2.3 Health of the lower gastrointestinal tract

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females. In 2008, there were an estimated 608,700 deaths due to the disease (Jemal *et al.*, 2011). Incidence rates of colorectal cancer have been increasing in countries in East Asia, Eastern Europe, and Western Europe such as Spain (Center *et al.*, 2009a, Center *et al.*, 2009b). However, incidence rates of colorectal cancer have been declining in the United States, Canada, and Australia (Center *et al.*, 2009a, Center *et al.*, 2009b).

The declining trend in incidence rates of colorectal cancer seen in the United States, Canada, and Australia may be due to a variety of modifiable factors including dietary patterns, smoking, and physical inactivity. Population-based screening in economically developed countries has played a role in reducing incidence rates. The United States is the only country with significantly decreasing incidence rates of colorectal cancer in both males and females in the last few years, possibly as a result of early detection and removal of precancerous lesions through colorectal cancer screening (Center *et al.*, 2009b). Rates of colorectal cancer

continue to increase in many countries with limited resources and health infrastructure (Center *et al.*, 2009a).

Data regarding the potential health benefits of whole grain consumption in the lower gastrointestinal tract, specifically with regards to colorectal cancer, vary between studies. This chapter will focus on the potential health benefit of whole grain consumption on the risk of colorectal cancer. Based on frequency of consumption, this chapter will also focus on the potential health benefit of wheat and rice consumption.

3. Proposed mechanisms for whole grain consumption and protection from colorectal cancer

Whole grains contain many phytochemicals; naturally occurring, non-nutrient compounds found in plants. These phytochemicals are generally thought to be responsible for the proposed association between increased whole grain consumption and reduced risk of colorectal cancer. One of the most studied class of whole grain phytochemicals is phenolic compounds. Phenolic compounds contain one or more aromatic rings and one or more hydroxyl groups (**Figure 1**). The predominant phenolic compounds found in whole grains and whole wheat are phenolic acids. Phenolic acids are hydroxybenzoic-acid and hydroxycinnamic-acid derivatives. Phenolic acids are generally found esterified or bound to cell wall polymers and are therefore insoluble when extraction solvents are used to extract phenolic compounds from whole or refined grains (Sosulski *et al.*, 1982). These compounds can be released from the cell wall by alkaline or acidic hydrolysis, or enzymatic activity. Andreasen *et al.* proposed a mechanism by which insoluble-bound phenolic compounds may be released in the lower gastrointestinal tract by gut microflora where they may exhibit potential health benefits (Andreasen *et al.*, 2001). For these reasons, the content of phenolic compounds in the insoluble-bound fraction of whole grains deserves the greatest attention when elucidating a mechanism for the association between whole grain consumption and reduced risk of colon cancer.

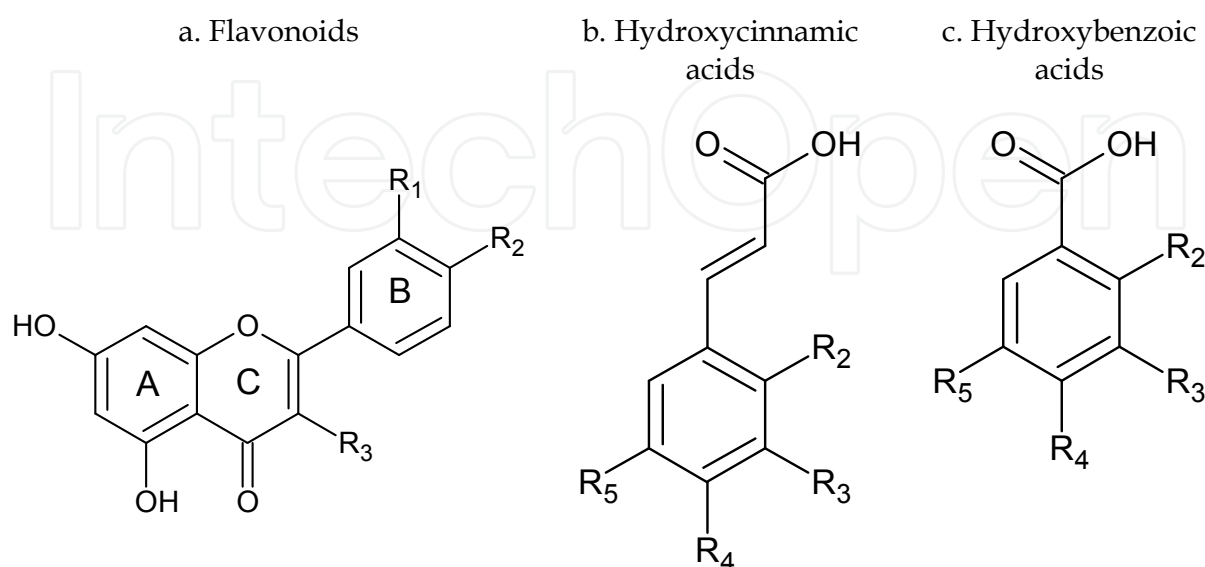


Fig. 1. Structure of phenolic acids and flavonoids

The multistage model for carcinogenesis involves the initiation of a normal cell, the promotion of an initiated cell to a preneoplasia, and finally the progression of the preneoplasia to an invasive tumor. The initiation of a normal cell can be caused by reactive oxygen species. Reactive oxygen species can oxidize biologically important molecules such as DNA.

3.1 Phenolic composition of the insoluble-bound fraction of whole grains

Many studies have determined the total phenolic content of the insoluble-bound fraction of whole grains. This is done using the total phenolic assay, which uses Folin-Ciocalteu Reagent (FCR). Initially, this assay was intended for the analysis of proteins taking advantage of the FCR's activity toward the amino acid tyrosine which has a phenol group. Singleton then used the assay to determine the content of total phenols in wine. The assay has since been used to determine the total phenolic content of fruits, vegetables, and grains. Phenolic compounds react with FCR under basic conditions only. Disassociation of the phenolic proton results in a phenolate anion, which is capable of reducing FCR (Huang *et al.*, 2005). The absorbance of the resulting solution is then measured at 750 nm and compared to the absorbance of various concentrations of a standard such as gallic acid to express the total phenolic content in terms of equivalents. Despite the undefined chemical nature of FCR, the total phenolic assay is convenient and reproducible.

The total phenolic content and phenolic composition of the insoluble-bound fraction of whole grains were determined in several studies. In these studies, the insoluble-bound phenolic compounds were extracted using solvents following alkaline hydrolysis. In one study investigating the total phenolic content and phenolic composition of grains, the total phenolic content of the insoluble-bound fraction of whole grains ranged from 24 (amaranth and buckwheat) to 255 (corn) mg gallic acid equivalents (GAE)/100 g. Ferulic acid and *p*-coumaric acid were found in the insoluble-bound fraction of all grain samples. Caffeic acid was only detected in the insoluble-bound fraction of barley and corn (4.2 and 1.8 $\mu\text{mol}/100$ g, respectively). Vanillic acid was only detected in the insoluble-bound fraction of quinoa. The insoluble-bound *p*-hydroxybenzoic acid content of amaranth and quinoa was 11.2 and 15.2 $\mu\text{mol}/100$ g, respectively. No flavonoids (quercetin, kaempferol, catechin, or rutin) or syringic acid were detected in the insoluble-bound fraction of whole grains.

The phenolic content and composition of the insoluble-bound fraction of grains is summarized below (Table 2).

The phenolic composition of a wide variety of grains and grain-based products was determined using reversed phase (RP)-high performance liquid chromatography (HPLC) with a diode array detector (DAD), (Mattila *et al.*, 2005). High levels of total phenolic acids were found in wheat bran (4527 mg/kg) and whole wheat flour (1342 mg/kg). The total phenolic acid content of refined wheat flour (167 mg/kg) was much lower than that of whole wheat flour (1342 mg/kg). White and brown rice samples had small amounts of free phenolic acids *p*-coumaric acid and ferulic acid. Parboiled rice contained mainly ferulic acid and *p*-coumaric acid (120 and 38 mg/kg, respectively). Roughly twice the amounts of ferulic acid and *p*-coumaric acid were found in brown rice (240 and 76 mg/kg, respectively).

The total phenolic content and phenolic acid composition of eight Maryland-grown varieties of soft wheat was determined using an RP-HPLC-DAD method (Moore *et al.*, 2005). Total

	Insoluble-Bound Phenolic Compounds					
	TPC†	FA*	<i>p</i> -CA*	<i>p</i> -HBA*	VA*	CA*
Corn	255 ± 5.6	558 ± 8.8	70.2 ± 2.4	nd	nd	1.8 ± 0.03
Wheat	122 ± 0.9	192 ± 15.7	12.1 ± 0.3	nd	nd	nd
Barley	94.1 ± 4.7	133 ± 12.2	19.0 ± 1.3	nd	nd	4.2 ± 1.6
Oats	79.5 ± 3.9	70.2 ± 7.4	26.4 ± 3.0	nd	nd	nd
Brown Rice	65.8 ± 0.7	88.6 ± 6.5	32.7 ± 0.8	nd	nd	nd
Quinoa	39.4 ± 2.9	35.5 ± 5.1	13.1 ± 1.0	15.2 ± 1.2	19.2 ± 11.2	nd
Amaranth	23.8 ± 0.3	6.5 ± 0.6	6.8 ± 0.2	11.2 ± 0.9	nd	nd
Buckwheat	23.5 ± 0.8	5.3 ± 0.2	6.3 ± 0.4	nd	nd	nd

†Total phenolic content values expressed as mg GAE/100 g.
*Phenolic compound content reported as µmol/100 g.
nd: not detected (detection limit of 160 µg/g of grain).
TPC, total phenolic content; FA, ferulic acid; *p*-CA, *p*-coumaric acid; *p*-HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CA, caffeic acid.

Table 2. The total phenolic content and phenolic acid content of the insoluble-bound fraction of whole grains.

phenolic content ranged from 40-80 mg GAE/100 g wheat. Vanillic, syringic, *p*-coumaric, and ferulic acids were found in all soft wheat samples. However, no *p*-hydroxybenzoic acid was detected in any of the soft wheat samples. Ferulic acid was the predominant phenolic acid found in all soft wheat samples. Similar to findings from other studies, most of the ferulic acid was found in the insoluble-bound fraction, ranging from 407-588 µg/g of grain, contributing between 89-95% of total ferulic acid content (84-90% of the total identified phenolic acids). The insoluble-bound fraction contributed the greatest proportion of *p*-coumaric acid for all soft wheat samples. Total phenolic content and contents of phenolic acid were not significantly correlated with antioxidant activity, suggesting that phenolic compounds were not predominantly responsible for the observed antioxidant activity. From this study, it was unclear which compounds were responsible for the observed antioxidant activity.

The total phenolic content and phenolic acid composition of the insoluble-bound fraction of six diverse varieties of whole wheat was determined using an RP-HPLC-DAD method (Okarter *et al.*, 2010). Ferulic acid was the predominant phenolic acid found in the insoluble bound fractions. The insoluble-bound ferulic acid content ranged from 272 (Caledonia) to 482 (KanQueen) µmol ferulic acid/100 g dry weight (DW). The bound ferulic acid content of KanQueen was significantly different (*p* < 0.05) from all other bound ferulic acid contents. The percentage of ferulic acid found in the insoluble-bound fraction ranged from 87.4 (Caledonia) to 97.2 % (KanQueen). *p*-Coumaric acid was also found in the insoluble-bound fraction of whole wheat. Insoluble-bound *p*-coumaric acid content ranged from 15.9 (Cham1) to 29.0 (KanQueen) µmol /100 g DW. The percentage of *p*-coumaric acid found in the insoluble bound fraction ranged 32.3 (Caledonia) to 63.4% (KanQueen). Syringic acid was found in the insoluble-bound fraction. Insoluble-bound syringic acid content ranged from 3.1 (Caledonia) to 9.8 (KanQueen) µmol /100 g DW. The insoluble-bound syringic acid content of KanQueen was significantly different (*p* < 0.05) from all other bound syringic acid contents. No insoluble-bound syringic acid was detected in the Foster variety. Caffeic acid

was only found in the insoluble bound fraction. Caffeic acid contents ranged from 3.2 (Caledonia) to 7.4 (KanQueen) $\mu\text{mol}/100\text{ g DW}$.

The total phenolic content and phenolic composition of the insoluble-bound fraction of two commercial blends of whole wheat and their refined flours were determined using an RP-HPLC-DAD method (Okarter, 2010). The total phenolic content of the insoluble-bound fraction of Magnolia and Barretta whole wheat was 95.8 and 97.5 mg GAE/100 g, respectively. The total phenolic content of the insoluble-bound phenolic fraction of Magnolia and Barretta refined wheat was 12.8 and 13.8 mg GAE/100 g, respectively. The insoluble-bound ferulic acid content of whole wheat ranged from 297-320 $\mu\text{mol}/100\text{ g}$. The insoluble-bound ferulic acid content of refined wheat ranged from 27-37 $\mu\text{mol}/100\text{ g}$. The insoluble-bound *p*-coumaric acid content of whole wheat ranged from 17-18 $\mu\text{mol}/100\text{ g}$. The insoluble-bound *p*-coumaric acid content of refined wheat ranged from 7-8 $\mu\text{mol}/100\text{ g}$. Caffeic acid was detected in the insoluble-bound fraction of whole wheat, but not refined wheat. The caffeic acid content of whole wheat ranged from 6-8 $\mu\text{mol}/100\text{ g}$.

3.2 Antioxidant activity of whole grains

Phenolic compounds are generally regarded as antioxidants. An antioxidant is a compound that can prevent or greatly retard the oxidation of easily oxidizable materials (Chipault, 1962). Antioxidants can transfer a hydrogen atom from a phenolic compound to the reactive oxygen species, preventing the oxidation of biologically important molecules. After transfer of the hydrogen atom to the reactive oxygen species, the antioxidant remains a stable compound by delocalizing the unpaired electron amongst the alternating single and double bonds. This process is known as hydrogen atom transfer. Antioxidants can also transfer an unpaired electron to an oxidant, resulting in the reduction of the oxidant. This process is known as single electron transfer. However, for phenolic compounds, free radical reduction by hydrogen atom transfer is preferred, as this requires lower energy (Leopoldini *et al.*, 2011). Antioxidant activity assays have been developed to determine the antioxidant activity of phenolic compounds using both processes.

3.2.1 Chemical antioxidant activity

Generally, antioxidant activity is assessed using a number of antioxidant activity assays. These assays involve the use of a free radical generator, a probe, and a standard. Though there is a large number of chemical antioxidant activity assays, the most common antioxidant activity assays are described below.

The Oxygen Radical Absorbance Capacity (ORAC) assay assesses the antioxidant activity of hydrophilic antioxidants (Cao *et al.*, 1993). Samples, controls, and a standard (e.g. Trolox, a hydrophilic analogue of vitamin E) are mixed with fluorescein solution and incubated at 37 °C before addition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) solution to initiate the reaction. The fluorescence intensity (excitation, 485 nm; emission, 525 nm) is measured every minute at ambient conditions (pH 7.4, 37 °C). As the reaction progresses, fluorescent intensity decreases. In the presence of phenolic compounds or phenolic extracts, the decrease in fluorescent intensity is prolonged. The decrease in fluorescent intensity is compared to that of Trolox and the antioxidant activity of the sample is then expressed as Trolox equivalents.

The Total Peroxyl Radical-Trapping Antioxidant Parameter (TRAP) assay uses a fluorescent probe (R-phycoerythrin) and free radical initiator (AAPH) to assess the antioxidant activity of phenolic compounds and phenolics extracts (Wayner *et al.*, 1985). As with the ORAC assay, the decrease in fluorescent intensity is prolonged in the presence of phenolic compounds or phenolic extracts. The decrease in fluorescence is monitored (excitation, 495 nm; emission, 575 nm) over time and compared to that of Trolox. The decrease in fluorescent intensity is compared to that of Trolox and the antioxidant activity of the sample is then expressed as Trolox equivalents.

The 2,2'-Azinobis(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) assay uses the ABTS•⁻ radical, which is dissolved in an aqueous potassium persulfate solution, resulting in a dark blue solution (Re *et al.*, 1999). This solution is then diluted with ethanol or buffer (pH 7.4) until a specific absorbance at 734 nm is reached. After addition of a standard, phenolic compounds or phenolic extracts, ABTS•⁻ is converted to ABTS²⁻ by single electron transfer resulting in a colorless solution. The decrease in absorbance is compared to that of Trolox, and the antioxidant activity of the sample is then expressed as Trolox equivalents. In this assay, Trolox or vitamin C can be used as a standard. When Trolox is used, the assay is referred to as the Trolox Equivalent Antioxidant Capacity (TEAC) assay. When vitamin C is used as the standard, the assay is referred to as the Vitamin E C Equivalent Antioxidant Capacity (VCEAC) assay. The concentration of phenolic compound or phenolic extracts giving the same percentage change of absorbance of the ABTS•⁻ as that of 1 mM Trolox is regarded as TEAC.

The Ferric Ion Reducing Antioxidant Power (FRAP) assay assesses the ability of Trolox, phenolic compounds, or phenolic extracts to reduce an Fe (III) salt to an Fe (II) salt (Benzie & Strain, 1996). After addition of Trolox, phenolic compounds or phenolic extracts, the Fe (III) is reduced to an Fe (II) salt. The absorbance of the resulting solution is measured at 593 nm. The resulting absorbance is compared to that after addition of Trolox and the antioxidant activity of the sample is then expressed as Trolox equivalents.

The antioxidant activity of grains was assessed using the TEAC, FRAP, and TRAP assays (Pellegrini *et al.*, 2006). Whole barley had the highest total antioxidant activity when using the TEAC and FRAP assays (obtained by the sum of soluble and bound compounds). Brown rice had the second highest total antioxidant activity when using the FRAP assay and spelt had the second highest total antioxidant activity when using the TEAC assay. However, spelt and brown rice had the highest total antioxidant activity when using the TRAP assay. White rice exhibited the lowest total antioxidant activity when using any of the three antioxidant activity assays. For all the grains analyzed the insoluble-bound fraction contributed to the majority of the total antioxidant activity. In the case of barley, brown rice, and spelt, the insoluble-bound fraction contributed 50% of the total antioxidant activity when using the TEAC assay. When using the TRAP assay, the insoluble-bound fraction contributed to all of the antioxidant activity of white rice.

The antioxidant activity of the insoluble-bound fraction of eight whole grains was determined using the ORAC assay (Okarter, 2010). The antioxidant activity of the insoluble-bound fraction ranged from 748 (amaranth) to 10089 (corn) μmol Trolox equivalents/100 g grain. The three pseudocereals had similar antioxidant activities in the insoluble-bound fraction. In this study, the total phenolic content of grains was correlated with antioxidant

activity ($R^2 = 0.880$, $p < 0.001$), suggesting that phenolic compounds were responsible for the observed antioxidant activity.

The total antioxidant activity of rice (white, red, and black) extracts was determined using the TEAC assay (Shen *et al.*, 2009). In this study, antioxidant activity was expressed as mM TEAC. Antioxidant activity ranged from 0.01 to 5.53 mM TEAC among the total rice accessions. Among the white rice, antioxidant activity ranged from 0.01 to 0.41 mM TEAC. Among the red rice, antioxidant activity ranged from 0.29 to 2.96 mM TEAC. The antioxidant activity of the black rice samples from this study was approximately three times that of the red rice. These data suggest that the phenolic compounds, which were responsible for the color of the rice, were also responsible for the observed antioxidant activity.

The antioxidant activity of eight Maryland-grown varieties of soft wheat was assessed using the ORAC and TEAC assay (Moore *et al.*, 2005). When using the TEAC assay, antioxidant activities ranged from 14.3 to 17.6 μmol of Trolox equivalents/g of soft wheat grains. The highest antioxidant activity was observed with the SS560 soft wheat line, and the least effective variety was Vigoro Tribute. There was significant variation between the wheat varieties when using the TEAC assay. Extracts from all soft wheat varieties or experimental lines exhibited significant ORAC values. When using the ORAC assay, antioxidant activity ranged from 32.9 μmol Trolox equivalents/g (Vigoro Tribute) to 47.7 μmol Trolox equivalents/g (Choptank).

The antioxidant activity of the insoluble-bound fraction of six diverse varieties of whole wheat was assessed using the ORAC assay (Okarter *et al.*, 2010). The ORAC ranged from 3190 (KanQueen) to 5945 (Roane) μmol Trolox equivalents/100 g DW. Total phenolic content was correlated with ORAC ($R^2 = 0.810$; $p < 0.001$).

The data regarding the antioxidant activity of the insoluble-bound fraction of whole grains varies between studies and are difficult to interpret due to the use of different antioxidant activity assays between studies and the lack of standardization between these antioxidant activity assays, the use of different grain varieties, and the different origin of the grain varieties between studies. This was clearly illustrated in the study that investigated the antioxidant activity of grains using three different antioxidant activity assays (Pellegrini *et al.*, 2006).

Antioxidant activity is correlated with total phenolic content in the vast number of studies investigating the antioxidant activity of grains. However, the correlation of total phenolic content with antioxidant activity may not be a meaningful correlation. Various phenolic compounds have varying antioxidant activities when assessed using the different antioxidant activity assays. For example, ferulic acid and *p*-coumaric acid have similar TEAC values (1.90 and 2.00, respectively). However, caffeic acid has a TEAC value of 1.00 even though its structure is similar to that of ferulic and *p*-coumaric acids. Further, the antioxidant activity of derivatives of hydroxycinnamates can vary significantly (Shahidi & Chandrasekara, 2010). Therefore, determining the phenolic composition of the phenolic extracts is also important.

3.2.2 Cellular antioxidant activity

The relevance of chemical antioxidant activity assays has been questioned due to the use of non-physiological temperature and/or pH, not accounting for bioavailability, uptake, or

metabolism (Fardet *et al.*, 2008, Wolfe & Liu, 2007). Wang *et al* (1999) developed a cell-based antioxidant activity assay, in which 2',7'-dichlorofluoresceine diacetate (DCFH-DA) is used to measure the loss of fluorescence upon quenching of the AAPH-induced reactive oxygen species by the pure compound or sample extract in HepG2 cell cultures (Wang & Joseph, 1999).

In this assay, HepG2 cells are on a 96-well microplate in growth medium/well. After seeding, the growth medium is removed and triplicate wells are treated for 1 h with 100 μ L treatment medium containing various concentrations of pure phenolic compound plus DCFH-DA. Following treatment, AAPH is applied to cells in 100 μ L Hank's Balanced Salt Solution (HBSS). Emission at 538 nm is measured with excitation at 485 nm every 5 min for 1 hour at 37°C. The concentrations used to determine the cellular antioxidant activity did not impact cellular viability, i.e. did not reduce the number of HepG2 cells by more than 10% compared to the control after 24 hours.

The cellular antioxidant activity of the phenolic compounds found in the insoluble-bound fraction of whole grains employing the DCFH-DA is reported below (Figure 2). Ferulic acid, the predominant phenolic acid found in whole grains, does not show any cellular antioxidant activity (Wolfe & Liu, 2007). Of the phenolic acids found in the insoluble-bound fraction of whole grains, only caffeic acid had any cellular antioxidant activity. All other phenolic acids did not show cellular antioxidant activity.

Another study aimed to complete the data regarding the cellular antioxidant activity of phenolic compounds found in the insoluble-bound fraction of whole grains (Okarter, 2010). None of the phenolic acids tested resulted in any cellular antioxidant activity. The cellular antioxidant activity of phenolic compounds is summarized below (Figure 2). Caffeic acid

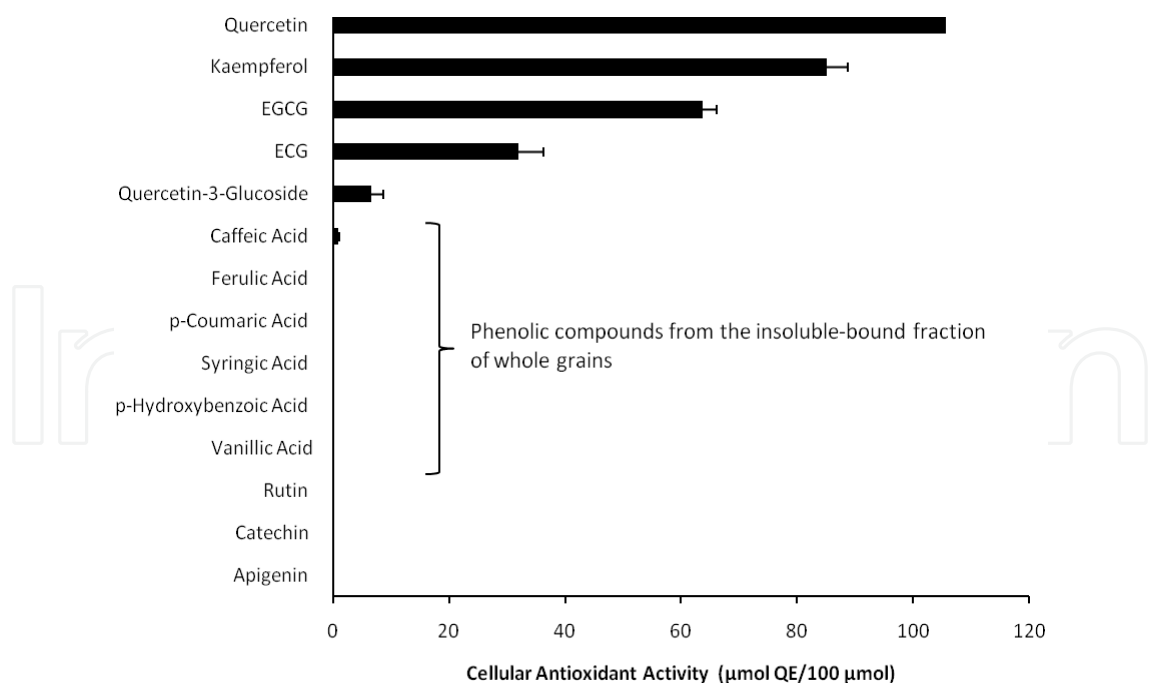


Fig. 2. Cellular antioxidant activity of phenolic compounds found in the insoluble-bound fraction (mean \pm standard deviation, n = 3) using the DCFH-DA assay. These data were pooled from several sources (Okarter, 2010, Wolfe & Liu, 2007, Wolfe & Liu, 2008). QE, quercetin equivalents; EGCG, epigallocatechin gallate; ECG, epicatechin gallate

was the only phenolic acid that had any cellular antioxidant activity. This may be due to the structure of caffeic acid. Caffeic acid is the only phenolic acid that has two hydroxyl groups located next to each other on the aromatic ring. Other phenolic acids may not have shown any cellular antioxidant activity due to the fact that these compounds were tested at concentrations that did not reduce the number of HepG2 cells by more than 10% compared to the control after 24 hours. At higher concentrations, these compounds may have measurable cellular antioxidant activity. However, the number of HepG2 cells remaining may not be enough to obtain a fluorescence signal. It may also be possible that phenolic compounds are absorbed to varying degrees by the HepG2 cell, further affecting their cellular antioxidant activity. The absorption of phenolic compounds by HepG2 cells was not assessed in any of these studies.

3.3 Antiproliferative activity of phenolic compounds and phenolic extracts from whole grains

Cell counting and cell proliferation assays are used to assess the ability of phenolic compounds or phenolic extracts to prevent or alter the proliferation of cell cultures *in vitro*.

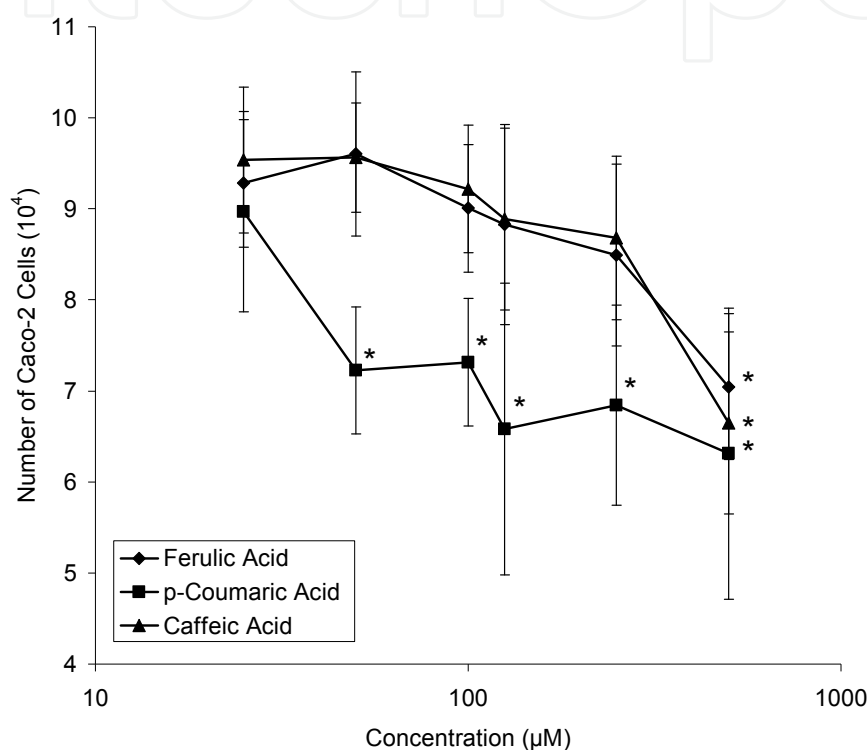
The Trypan Blue Stain Assay is the most common cell counting assay. This assay involves direct cell counting after staining cells with trypan blue staining followed by microscopic quantification using a hemacytometer. This process involves trypsinizing adherent cells, removing cells from culture, centrifugation and re-suspension, staining with trypan blue, and cell counting. Loss of cells due to trypsinization and the resulting degradation of chromatin are sources of underestimated cell numbers.

The Methylene Blue Stain Assay is another common cell counting assay (Oliver *et al.*, 1989). Methylene Blue is a basic dye that is positively charged at pH 5-8. It binds electrostatically to negatively charged groups within cells, predominately phosphate moieties of nucleic acids and some charged groups in proteins. This explains the variation in dye bound by cells from different species, and also by different cell types within the same species. Lowering the pH below 2 with HCl causes acidic groups to be protonated, liberating the Methylene Blue into the elution solvent. The absorbance of the resulting solution is assessed at 570 nm and fit to a standard curve to determine cell number.

The Microculture Tetrazolium Salt (MTS) assay is based on the conversion of a tetrazolium salt into a colored, aqueous soluble formazan product by mitochondrial activity of viable cells at 37°C. The amount of formazan produced by dehydrogenase enzymes is directly proportional to the number of living cells in culture and can be measured at 492 nm.

The antiproliferative activity of phenolic acids and phenolic extracts from brown rice was determined in three different colon cell lines using the MTS assay (Hudson *et al.*, 2000). Caffeic acid (50 µM) inhibited the proliferation of all three colon cell lines (HT29, SW480, and HCEC) used in the study. Methoxycinnamic acid inhibited the proliferation of two of the colon cell lines used in the study. Ferulic acid and sinapic acid inhibited the proliferation of only one cell line (HCEC and SW480, respectively). *p*-Coumaric acid did not have any effect on cell proliferation in any of the three colon cell lines. Phenolic extracts from brown rice inhibited the proliferation of two out of the three colon cell lines used in the study (SW480 and HCEC).

The antiproliferative activity of phenolic acids and phenolic extracts from the insoluble-bound fraction of two commercial blends of whole wheat and their refined flours was determined using the Methylene blue stain assay (Okarter, 2011). *p*-Coumaric acid (50 μ M) significantly ($p < 0.05$) reduced the number of Caco-2 cells at 96 hours post-treatment compared to the number of cells when cells were grown under control growth conditions (**Figure 3**). Ferulic acid and caffeic acid significantly ($p < 0.05$) reduced the number of Caco-2 cells at 96 hours post-treatment at a concentration of 500 μ M compared to the number of cells when cells were grown under control growth conditions (**Figure 3**). The concentrations of phenolic acids used in this study are achievable through diet (Janicke *et al.*, 2005).



* indicates significant difference from the number of Caco-2 cells when cultured under control growth conditions (no phenolic acids added; $p < 0.05$).

Fig. 3. Number of Caco-2 cells after 96 hours of treatment with phenolic acids (mean \pm standard deviation, $n = 3$)

Phenolic extracts from the insoluble-bound fraction of whole wheat but not refined wheat significantly inhibited the proliferation of Caco-2 cells at 24 and 96 hours post-treatment, compared to cells cultured under control growth conditions (**Table 3**).

In another study, the effect of ferulic and *p*-coumaric acids on Caco-2 cell proliferation was assessed using the Trypan Blue stain assay (Janicke *et al.*, 2005). Ferulic acid and *p*-coumaric acid treatment significantly inhibited the proliferation of Caco-2 cells at 72 hours post treatment at a concentration of 1500 μ M.

Results from these studies suggest that the phenolic compounds found in the insoluble-bound fraction of whole grains protect against colon cancer by inhibiting the proliferation of cells.

	24 hours	96 hours
	Cell number (10 ⁴)	Cell number (10 ⁴)
Medium Control	7.3 ± 0.7	9.0 ± 0.8
Solvent Control ‡	7.6 ± 0.6 (104.2%)	9.5 ± 0.9 (104.2%)
Refined Wheat †		
Barretta	7.1 ± 1.3 (96.6%)	8.8 ± 1.1 (97.8%)
Magnolia	7.0 ± 1.6 (96.3%)	8.9 ± 0.9 (99.6%)
Whole Wheat †		
Barretta	4.6 ± 0.2 (59.9%) *	7.0 ± 2.1 (76.6%) *
Magnolia	4.5 ± 1.2 (59.0%) *	7.1 ± 1.4 (78.2%) *

‡ 10% v/v sterile water in growth medium; † Phenolic extracts were delivered at a grain sample concentration of 100 mg/mL
*Within each column indicates a significant difference from the medium control at p < 0.05

Table 3. Effects of phenolic extracts from the insoluble-bound fraction of whole and refined wheat on Caco-2 cell number 24 and 96 hours post-treatment. Values are reported as number of Caco-2 cells (mean ± standard deviation, n = 3). Percent proliferation of the medium alone (control) is in parentheses.

4. *In vivo* data regarding whole grain consumption and health of the lower gastrointestinal tract

Given the amount of *in vitro* data, supported by proposed mechanisms for the association between whole grain consumption and cancer development, we do not see these clear associations with colon cancer development *in vivo*. Gene activation or changes in intracellular signaling cascades may partially explain why we do not see these associations *in vivo*.

4.1 Animal studies

One study investigated the bioavailability of ferulic acid in rats fed a ferulic acid-supplemented diet (Adam *et al.*, 2002). The amount of ferulic acid in the feces was negligible regardless of the amount of ferulic acid that was added to the diet suggesting that ferulic acid is absorbed by the gastrointestinal tract or metabolized by gut microflora in the cecum. In plasma, ferulic acid was detected only after enzymatic treatment (5 × 10⁶ units/L β-glucoronidase and 2.5 × 10⁵ units/L sulfatase), suggesting that ferulic acid is circulated only as conjugates. Ferulic acid was not detected in the plasma 18 h after consuming a meal supplemented with ferulic acid suggesting it is either poorly absorbed or quickly eliminated following absorption via urinary excretion. Fecal excretion of ferulic acid was enhanced following its addition to a high wheat semi-purified diet, suggesting that the absorption of this phenolic acid is decreased when present in a complex matrix such as cereals. These data suggest that the cereal matrix severely limits the bioavailability of ferulic acid in rats, and imply a significant role for gut microflora in ferulic acid bioavailability.

Another study investigated the effects of brown rice, rice bran, and polished rice on preneoplastic lesions of the colon in rats (Li *et al.*, 2011). Consumption of brown rice, rice bran, or polished rice had no effect on plasma or hepatic thiobarbituric acid reactive substances (TBARS), a measure of antioxidant activity. Further, consumption of brown rice,

rice bran, or polished rice did not affect the number of mucin-depleted foci (MDF), preneoplastic lesions of colon cancer. Consumption of rice bran significantly reduced the number of 2-crypt aberrant-crypt foci (ACF), another preneoplastic lesion of colon cancer, compared to rats consuming the control diet ($P < 0.05$). Most ACF were observed in the middle colon. Consumption of rice bran (AIN-93G containing 2.2% rice bran) significantly reduced the number of ACF in the middle colon ($P < 0.05$). Interestingly, the number of ACF in the distal colon was significantly increased in rats fed a diet containing 17.8% polished rice compared to rats fed a control diet ($P < 0.05$). COX-2 protein expression in the middle colon was significantly lower in all rats fed experimental diets compared to rats fed a control diet ($P < 0.05$). There were no significant differences in COX-2 protein expression among all treatment groups in the proximal and distal colon.

The data from these animal trials suggest that ferulic acid and perhaps other hydroxycinnamic acids obtained from food have no effect on antioxidant measures and the formation of other markers of colon cancer. This may be because the food matrix affects the bioavailability of these hydroxycinnamic acids, and phenolic acids are metabolized by gut microflora.

4.2 Human studies

Many clinical studies have investigated the effects of whole grain consumption on various health-related outcomes or incidence of colorectal cancer. These studies are summarized below.

One study concluded that absorption of hydroxycinnamic acids present in cereals is limited (Kern *et al.*, 2003a). Six healthy volunteers underwent a 2-day low phenolic compound diet prior to the study day that avoided bran cereals, whole grain products, seeds and nuts, fruits and vegetables, herbs and spices, and other products. The volunteers were allowed to eat foods low in phenolic compounds (white bread, white pasta, white rice, etc). On the study day and immediately before eating the test meal a blood sample (30 mL) was taken as a baseline control. Following blood sampling, volunteers consumed 100 g of a commercial breakfast cereal (85% wheat bran) with skimmed milk. Subsequent blood samples (30 mL each) were taken at various times after the test meal. Low-phenolic compound meals and water were served during the study day. Volunteers collected urine for 24 h on the day prior to the study day and throughout the study day. The researchers found that ferulic acid and sinapic acid were the major hydroxycinnamic acids taken up in humans after the consumption of a high-bran cereal. Further, maximum levels of these compounds reached in plasma were in the nanomolar range, though the content in food was in the micromolar range. The researchers concluded that absorption of the compounds occurred mostly from the small intestine and that the bulk of ester-linked dimeric compounds were excreted in feces or further metabolized by colonic microflora.

Another study reported that there were no effects on antioxidant measures (oxygen radical absorbance capacity in blood, and isoprostane and thiobarbituric acid reactive substances in urine) after 14 days of consumption of whole grain or refined grain food products in healthy subjects (mean age, 27.1 years; mean BMI, 23.9 kg/m²) (Enright & Slavin, 2010). The study was a randomized, crossover design with two 14-day intervention periods (whole grain or refined grain) with no washout period in between. Subjects were assigned to either a diet

containing eight servings for men and six servings for women of whole grain foods in addition to their regular diet or a diet containing eight servings for men and six servings for women of refined grain foods in addition to their regular diet. After 14 days, they were switched to the other group. The results from this study suggest that higher total phenolic content of test meals, higher *in vitro* antioxidant activity, and the presence of more phenolic compounds, as found in whole grains compared to refined grains, do not correlate with increased antioxidant activity *in vivo*.

Epidemiological studies have also investigated the association between whole grain consumption and reduced risk of colorectal cancer. Results from these studies are described below (**Figure 4**), suggesting a slight but often not significant positive health effect on rectal and colorectal cancer upon consumption of whole grains.

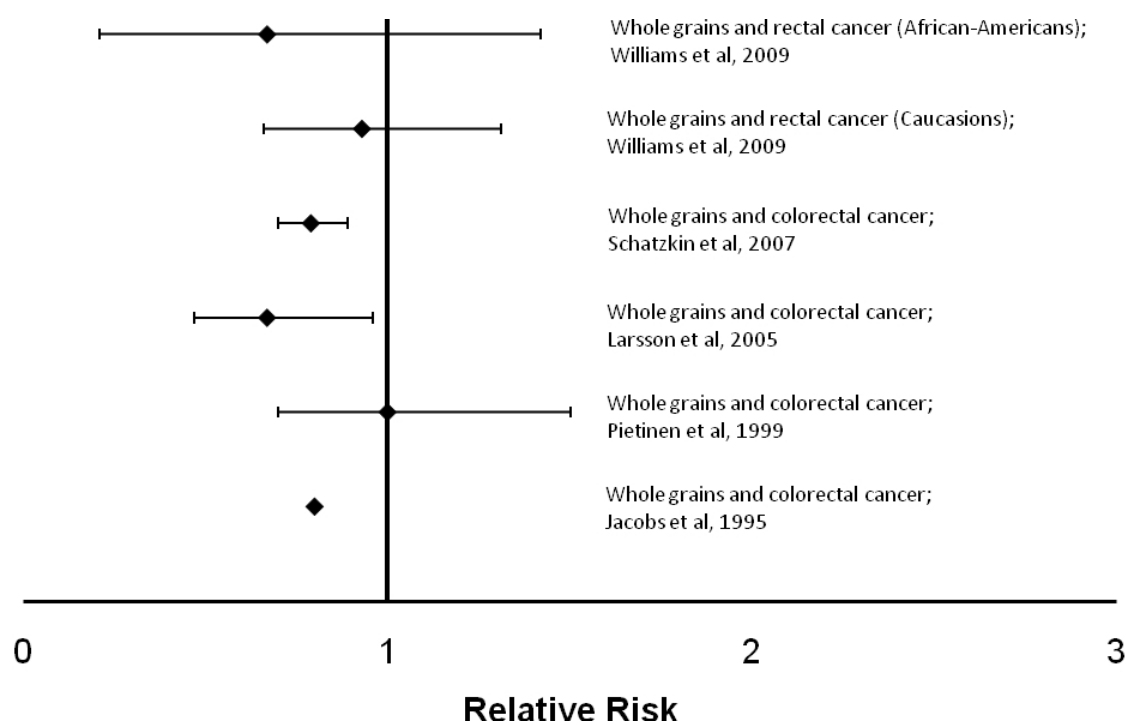


Fig. 4. The association between whole grain consumption and reduced risk of colorectal cancer according to several human studies (relative risk ± 95% confidence interval)

Researchers from Scandinavia investigated the association between cereal consumption and risk of colorectal cancer using data from the Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study (Pietinen *et al.*, 1999). Subjects were male smokers aged 50-69 years who were recruited from the total male population of the same age range in southwestern Finland. Diet was assessed at baseline using a self-administered modified dietary questionnaire. The questionnaire included 276 food items and a portion size picture booklet with photographs of foods, each with 3-5 different portion sizes. Food consumption data were processed using the software and food composition database provided by the National Public Health Institute. After adjusting for confounding factors including years of smoking, BMI, physical activity level and education, there was no significant association between whole grain cereal consumption and risk of colorectal cancer, when comparing the highest quartile of whole grain cereal consumption to the lowest.

One study investigated the association between whole grain consumption and colon cancer using data from the Swedish Mammography Cohort. Dietary information was obtained from a 67-item food-frequency questionnaire administered at baseline. Incidence of colorectal cancer was obtained after a mean of 14.8 years. After adjusting for red meat, fruit, and vegetable consumption and other possible confounding factors, whole grain consumption was inversely associated with risk of colon cancer ($RR = 0.67$; 95% $CI = 0.47 - 0.96$) when comparing the highest quintile of whole grain intake (≥ 4.5 servings/day) to the lowest (< 1 serving/day) (Larsson *et al.*, 2005).

Another study investigated the association between whole grain consumption and colorectal cancer using data from the NIH-AARP Diet and Health Study. The diet of nearly 490,000 subjects aged 50-71 years was assessed with a self-administered food-frequency questionnaire at baseline in 1995-1996. During the 5 year follow up, 2974 incident colorectal cancer cases were identified. The researchers found that there was an inverse association between whole grain consumption and risk of colorectal cancer ($RR = 0.79$; 95% $CI = 0.70 - 0.89$) when comparing the highest quintile of whole grain intake (1.3 servings/1000 kcal/day) to the lowest (0.2 servings/1000 kcal/day) after multivariate analysis (Schatzkin *et al.*, 2007). This association was stronger for men ($RR = 0.79$; 95% $CI = 0.68 - 0.91$) than for women ($RR = 0.87$; 95% $CI = 0.70 - 1.07$). The association between whole grain consumption and reduced risk of site specific tumors was strongest for the rectum ($RR = 0.64$; 95% $CI = 0.51 - 0.81$).

Researchers from the United States investigated the association between whole grain consumption and risk of rectal cancer in both Whites and African-Americans using data from the North Carolina Cancer Study - Phase II. The dietary intake of 1520 Whites and 384 African-Americans was assessed using the Diet History Questionnaire, which consisted of 124 separate food items and assessed the frequency of consumption and portion size consumed for each food item. Participants estimated their food and beverage intake from the 12 months prior to the study. After adjusting for age, non-steroidal anti-inflammatory drug use, total energy, and other possible confounding factors, whole grain consumption was not associated with reduced risk of rectal cancer in White-Americans ($RR = 0.93$; 95% $CI = 0.66-1.31$) or African-Americans ($RR = 0.67$; 95% $CI = 0.21-1.42$) when comparing the highest quartile of whole grain intake to the lowest (Williams *et al.*, 2009).

Thus, the findings regarding the potential health benefits of whole grain consumption in the lower gastrointestinal tract are mixed. Some studies show an association between whole grain consumption and reduced risk of colorectal cancer while some studies show no association. The difference in findings may be due to the study design, sample population, type of whole grains consumed from baseline to follow-up, and/or other possible factors.

5. Barriers to potential health benefits in the lower gastrointestinal tract

There are many possible factors that may affect the ability of phenolic compounds from the insoluble-bound fraction of whole grains to provide any health benefits in the lower gastrointestinal tract. These factors are discussed in further detail below.

5.1 Effect of processing on phenolic composition

It is important to recognize that human beings rarely eat whole grains unprocessed. Human beings eat food products made from whole and/or refined grain flours. The various food processing methods that occur prior to consumption of grain-based food products (kneading, baking, puffing, fermenting, extruding, boiling, nixtalmizing, etc.) may alter the total phenolic content and/or phenolic composition of the insoluble-bound fraction of the grain.

One study investigated the effect of processing on phenolic acid content of grain-based food products using an RP-HPLC-DAD method (Mattila *et al.*, 2005). Baking, cooking, or other processing did not destroy phenolic acids. The total phenolic acid content of traditional and precooked oat flakes was identical. The contents of phenolic acids in rye and wheat flours and corresponding products made from these flours (bread and pasta) were found to be similar as well. There were only minor differences when comparing the phenolic acid contents of organic and conventional rye and wheat flours.

Another study investigated the effect of cooking and processing on the phenolic acid profile of bran enriched pastas (Fares *et al.*, 2010). Processing decreased the content of free phenolic acids by nearly 50%. However, the phenolic acid content of the insoluble-bound fraction did not change after processing. The total phenolic acid content decreased after processing, possibly as a result of the oxidative degeneration that occurs due to the addition of water, heat treatment, and kneading during processing. Cooking generally increased the content of phenolic acids found in the insoluble-bound fraction of all samples to varying degrees (36-87%). Pasta had significantly enhanced *in vitro* antioxidant properties after cooking, most likely because cooking releases esterified phenolic compounds from the plant cell wall and leads to the formation of Maillard reaction products, which have measurable antioxidant activity.

The effect of fermentation on the phenolic content and antioxidant activity of cereals (buckwheat, barley, wheat, and rye) was assessed using the FRAP assay (Dordevic *et al.*, 2010). Fermentation led to increase in the total phenol content of all four cereals by up to 39%. Antioxidant activity was increased when samples were fermented with *L. rhamnosus* compared to samples fermented with *S. cerevisiae*. Fermentation did not have any effect on antioxidant activity assessed using the FRAP assay.

Another study investigated the difference in antioxidant activity between pasta made from whole wheat compared to pasta made from refined wheat (Hirawan *et al.*, 2010). The total phenolic content of refined wheat and whole wheat spaghetti were significantly different before and after cooking. Cooking significantly decreased the total phenolic content of refined wheat and whole wheat spaghetti by 22-53%. The average antioxidant activity, assessed using the ORAC assay, was not significantly different between refined wheat spaghetti and whole wheat spaghetti.

5.2 Metabolism of phenolic acids

One study investigated the formation of conjugates and metabolites from hydroxycinnamics (Kern *et al.*, 2003b). The human small intestine epithelium may contribute to the metabolism and bioavailability of hydroxycinnamates. Methyl-cinnamate-sulfate and methyl-cinnamate glucuronide conjugates were the main metabolites formed after metabolism of

hydroxycinnamic acids in Caco-2 cells. These results suggest that sulfation may be the preferred metabolic pathway for hydroxycinnamic acids in the small intestinal epithelium. The glucuronide derivatives of hydroxycinnamic acids may be products of liver metabolism.

The products of microfloral metabolism of dietary phenolic compounds were previously summarized (Aura, 2008). Ferulic acid can be transformed to 3-(3-hydroxyphenyl) propionic acid (Aura, 2008). Caffeic acid can be transformed either to 4-ethylcatechol or to 3-(3-hydroxyphenyl) propionic acid by human fecal microflora (Peppercorn & Goldman, 1971). Quercetin can be transformed to 2-(3-hydroxyphenyl) acetic acid by a process called ring fission (Aura *et al.*, 2002). Other hydroxycinnamates and flavonoids may be metabolized by the intestinal microflora to similar structures.

5.3 Uptake of phenolic acids from the intestinal lumen

The monocarboxylic acid transporter (MCAT) is an active transporter located on the apical and basolateral sides of the Caco-2 cell. Transport of hydroxycinnamates from the intestinal lumen to the plasma involves the simultaneous transport of hydrogen ions. Studies have shown that the MCAT is involved in the transport of hydroxycinnamates and their metabolites into epithelial cells (Konishi *et al.*, 2003, Konishi & Kobayashi, 2004, Konishi, 2005, Konishi & Kobayashi, 2005).

Other research in this field suggests that the MCAT may not be involved in the uptake of phenolic acids from the lumen of the gastrointestinal tract (Watanabe *et al.*, 2006). The uptake of phenolic acids from the intestinal lumen may occur via the nateglinide/H⁺ active transport system (Itagaki *et al.*, 2005). One study showed that phenolic acids (caffeic acid, *p*-coumaric acid, and chlorogenic acid) have different affinities for this transporter as demonstrated by the ability to inhibit nateglinide uptake (Saito *et al.*, 2005).

Research data regarding the uptake of phenolic acids from the intestinal lumen are mixed. However, the existing data do show that phenolic acids have varying affinities for the transporters used to transport phenolic acids into the intestinal epithelium. It is important to know and understand the varying affinities of hydroxycinnamate metabolites and conjugates in order to understand the potential of phenolic acids from the insoluble-bound fraction to impart potential health benefits in the lower gastrointestinal tract.

6. Conclusion and future research

Whole grain consumption has been associated with reduced risk of cardiovascular disease, type 2 diabetes, and obesity. However, data regarding the association between increased whole grain consumption and reduced risk of colorectal cancer are mixed. The potential health benefits of whole grain consumption in the lower gastrointestinal tract may be due to the content of phenolic compounds; compounds with one or more aromatic ring and one or more hydroxyl group. The phenolic content and composition of whole grains have been reported on several occasions. Generally, phenolic compounds are found esterified to cell wall polymers, which enables them to survive digestion in the upper gastrointestinal tract, and allows them to impart their potential health benefit in the lower gastrointestinal tract.

Phenolic compounds are believed to impart health benefit, at least in part, due to their antioxidant activity. Generally, antioxidant activity is assessed using a number of *in vitro*

chemical antioxidant activity assays. However, these assays do not take into account factors such as bioavailability and metabolism. When *in vitro* cellular antioxidant activity assays are used to assess the antioxidant activity of phenolic compounds found in grains, data show that these compounds do not have any antioxidant activity, except for caffeic acid. These data suggest that any potential health benefit of whole grain phenolic compounds is independent of antioxidant activity.

Phenolic compounds found in whole grains have the ability to inhibit the proliferation of colon cells *in vitro*. Further, phenolic extracts from whole wheat and brown rice inhibit the proliferation of colon cells *in vitro*. Scientific evidence suggests that other pathways such as intracellular signaling, cell cycle arrest, and apoptosis are the major reasons for the observed effect of treatment with phenolic compounds on cell proliferation (Hou *et al.*, 2004, Janicke *et al.*, 2005, Romier *et al.*, 2008).

There are several factors to take into account when considering the potential health benefits of whole grain phenolic compounds in the lower gastrointestinal tract. The processing of whole grains affects the content of phenolic compounds found in the insoluble-bound fraction of whole grains. Further, intestinal microflora metabolize these phenolic compounds, further reducing their contents. Remaining phenolic compounds and their metabolites must then compete for transport into the cell via the MCAT, the nateglinide/H⁺ transport system, or other similar transport systems.

Further research in the field of whole grain consumption and health of the lower gastrointestinal tract must consider all the factors that affect the content of phenolic compounds including food processing, microfloral metabolism, uptake into the intestinal epithelium, and the effect of these compounds on the proliferation of colon cells. Other phytochemicals and nutrients found in grains (carotenoids, vitamin E, alkylresorcinols, and γ -oryzanols) and dietary fiber and soluble phenolic compounds that survive digestion in the upper gastrointestinal tract also contribute to the health benefits of whole grain consumption. The use of animal models provides valuable insights but must also consider the composition of the diet given to test animals as well as the microfloral content, composition, and activity of the animals' gastrointestinal tract. Epidemiological studies should focus more on and report more detailed descriptions of the grains consumed by subjects. Prospective or intervention studies could answer some aspects on the benefits of grain on the colon.

Whole grains play an important role in reducing the risk of chronic disease. More work is needed to understand the potential health benefits of whole grains in the lower gastrointestinal tract.

7. References

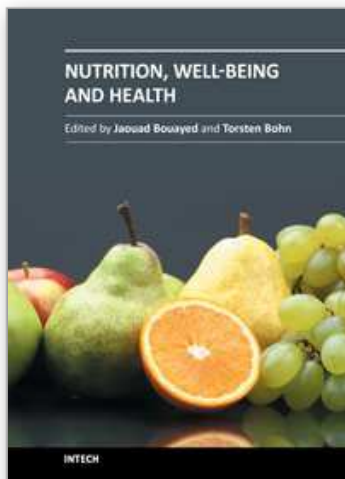
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Nutrition, Well-Being and Health

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In our modern society, expectations are high, also with respect to our daily diet. In addition to being merely "nutritious", i.e. supplying a variety of essential nutrients, including macro-nutrients such as proteins or micro-nutrients such as minerals and vitamins, it is almost expected that a good diet offers further advantages - especially well-being and health and the prevention of chronic diseases, which are, as we generally tend to grow older and older, becoming a burden to enjoying private life and to the entire society. These additional qualities are often sought in diets rich also in non-nutritive components, such as phytochemicals. In contrast to drugs, which are taken especially to cure or ameliorate diseases, it is expected that a healthy diet acts in particular on the side of prevention, allowing us to become old without feeling old. In the present book, rather than trying to give an exhaustive overview on nutritional aspects and their link to well-being and health, selected topics have been chosen, intended to address presently discussed key issues of nutrition for health, presenting a reasonable selection of the manifold topics around diet, well-being, and health: from the antioxidants polyphenols and carotenoids, aroma-active terpenoids, to calcium for bone health, back to traditional Chinese Medicine.

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