PROVISION OF WHOLE GRAINS TO MIDDLE SCHOOL STUDENTS ACHIEVES DIETARY GUIDELINES FOR AMERICANS AND MYPLATE RECOMMENDATIONS

By

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To my parents

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I thank my parents, family, friends, and mentors Dr. Dahl and Dr. Henken for guiding and supporting me through this process. The knowledge and experiences I have gained are life changing.

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Middle school students should consume 5 to 6 oz equivalents/d (girls) and 6-7 oz equivalents (boys) of grains, with at least half as whole grain according to the United States Department of Agriculture's (USDA) dietary guidelines. Current total intakes average 5.3 oz equivalents/d, with whole-grain intakes at 1.1 oz equivalents/day. The aim of this study was to determine the effect of providing refined or whole-grain foods to adolescents with encouragement to eat three different grain-based foods per day, on total grain and whole-grain intakes. Middle school students (n=83; 11-15 y) were recruited from Westwood Middle School, Gainesville FL, and randomized to refined- or whole-grain foods for 6 weeks. The adolescents and their families were provided with weekly grains (e.g. bread, pasta, and cereals), and participants were provided grain snacks at school. Two baseline and 6 intervention targeted 24-hr diet recalls were carried out, and intakes in ounces were determined. Total grain intake for males increased when provided refined grains (6.0±0.5 to 7.2±0.4 oz equivalents/d; $p<0.05$), and whole-grain foods (5.0±0.5 to 6.8±0.5 oz equivalents/d; $p<0.001$). Total grain intake for females did not change with either intervention. When whole-grain foods were provided, whole-grain intake increased for males (1.0±0.2 to 4.2±0.4 oz equivalents/d; $p<0.0001$) and females (0.9±0.3 to 3.5±0.3 oz equivalents/d; $p<0.0001$). In conclusion, providing adolescents with whole-grain foods is an effective means of achieving recommendations.

CHAPTER 1
LITERATURE REVIEW

Whole Grain

Whole Grain Definition

Whole grains have been a part of the human diet for about 10,000 years (1). In fact, the majority of the world's population has relied on whole grains as a main component of the diet. In the United States, 66-75% of the total grain consumed is from wheat (2). Whole wheat, oatmeal, maize, brown rice, whole-grain barley, whole rye, and millet are all examples of whole grains grown and consumed around the world (2). The definition of a whole grain is currently a disputed topic in the scientific community.

In the United States, a uniform definition of what constitutes a whole grain is established, however internationally, there is no accepted definition of whole grain. The U.S. Food and Drug Administration (FDA) defines a whole grain as consisting of the intact, ground, cracked, or flaked kernel (also known as the caryopsis, seed or fruit), which includes the bran, the germ, and the inner most part of the kernel (the endosperm). This definition has been adapted from the American Association of Cereal Chemists (AACC). The Dietary Guidelines for Americans 2010 describe a whole grain as consisting of the entire grain seed, usually called the kernel. They define the kernel as consisting of three components: the bran, germ, and endosperm. If the kernel has been processed (cracked, crushed, or flaked), then to be called a "whole grain" the food must retain the same relative proportions of the previously listed components, as they exist in the intact grain. Another definition comes from the American Whole Grain Council (WGC): "Whole grain or foods made from them contain all the essential parts and naturally occurring nutrients of the entire grain seed. If the grain has been processed (e.g. cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same rich balance of nutrients that are found in the original grain seed." All of these definitions are similar in that they define a whole grain based on composition (consisting of the bran, germ, and wheat) and the raw material of the grain (ground, cracked, or flaked) (3).

As of 2010, the definition of a whole grain is different all around the world. Many researchers agree that one, strict and uniform definition of whole grain has not been established or accepted internationally and is urgently needed (4, 5). Cummings et al. (5) suggest that, the term whole grain be more clearly defined, concentrating on the role of intact versus milled grains. Some definitions are very broad, for example the AACC and FDA definition in the United States includes 22 cereals and pseudocereals, while a Danish Task Force (4) defines whole grain as intact, ground, cracked, or flaked kernels after removal of the husks which only includes nine cereals as whole grain. Denmark, Sweden, and Norway agreed upon a definition of whole grain to include intact and processed products where endosperm, germ, and bran are present in the same proportion as in the intact grain. The definition goes on to describe that if fractions were to be separated under processing, they should be added back so that the final product has the same proportions of the three parts as in the intact grain. Some whole grains that fall under this definition include whole grain cereals: wheat, rye, oats, barley, maize, rice millet and sorghum while omitting wild rice, quinoa, amaranth, and buckwheat (4). Depending on the definition, anywhere from 7 to 22 different grains may be included.

De Moura et al. (6) explain that using the FDA definition of whole grain as a selection measure is limiting due to the fact that the majority of existing studies often use an even broader meaning to define whole grain. In a review of studies on the association of whole grain intake and incidence of cardiovascular disease (CVD), only four of the 204 articles evaluated conformed to the FDA definition of whole grain. Most of the scientific literature uses a broader concept of whole grain, such as looking at just the effects of bran to draw conclusions about whole grains (6).

In 2010, Frolich et al. (4) explain the need for a universally agreed upon definition of whole grain. This has proven to be difficult due to the diversity of whole grain composition in terms of macronutrients, micronutrients, and phytochemicals. The author goes on to explain that only those grains which have similar nutrient content should be included as a whole grain. Much of the issue surrounds which type of seed should be included in the definition (4).

Currently there are three groups of “whole grains” or seeds in question. The first group is cereals, a member of the grass family. Cereals produce dry, one-seeded fruits (caryopsis), which are commonly known as the kernel or grain. All cereals have generally similar compositions made up of the bran, germ, and endosperm. Examples of cereals include wheat, rice, oats, and barley. Next is the pseudocereal group, which is not a member of the grass family. These plants have high starch content in their seeds and so are used in cereal-like products. Some examples of pseudocereals are amaranth, quinoa, and buckwheat. Last, is the pulse or grain legume group, which is not often regarded as whole grain, but because of their high-starch content, may fall into the definition of a whole grain (4).

Frolich et al. (4) suggest that a definition for whole grain be formulated using two steps: first, by agreeing on a definition regarding the raw and dry materials (milled, cracked, crushed, rolled, or flaked) and secondly, agreeing on a definition in terms of the preparation process (thermal, enzymatic, baking, malting, and fermentation). Okarter et al. (7) suggest defining whole grain based on the composition. The author explains that a whole grain can have its original composition of the bran, germ, and endosperm intact or can be a reconstituted whole grain product in which the original components are recombined to the same proportion as the naturally occurring grain kernel. Taking these suggestions from the authors into consideration, the grain in question can then be tested for health effects and defined as a whole grain (4).

Using a universal definition for whole grain is important because much of the existing literature cites a relationship between whole grain consumption and health benefits. Unfortunately, the lack of a general definition of whole grain for dietary assessment raises the question of validity and accuracy concerning the amount of whole grain consumed in research studies. Some studies include products containing added bran and germ, so-called pseudocereals, partial whole grain products, and starch-rich seeds. In conclusion, Frolich et al. argue that finding an applicable definition of whole grain will be helpful for individual consumers to facilitate their choice of healthy food alternatives; the scientific community, to establish a uniform dietary factor to study in relation to health; the authorities, to allow them to make recommendations; or the food industry, to produce new whole grain products (4).

Under the current FDA, WGC, and AACC definitions of whole grain, the following cereals and pseudeocereals are considered whole grain: amaranth, barley, buckwheat, corn (including whole cornmeal and popcorn), millet, oats, including oatmeal and quinoa, rice (both brown rice and colored rice), rye, sorghum, teff, triticale, wheat (including varieties such as spelt, emmer, faro, einkorn, kamut, durum, and forms such as bulgur, cracked wheat and wheatberries), wild rice, canary seed, Job's tears, montina, and fonio. Under the whole grain definitions in the United States, oilseeds and legumes are not considered whole grain (4).

The FDA allows health claims on products, which have scientific backing, to help the consumer make better food choices. In order for a product to bear the whole grain claim, the FDA requires that the food contain 51% or more whole grain ingredients by weight per reference amount customarily consumed (RACC). In order to assess compliance of a product with the FDA regulation it should be compared to the dietary fiber level of whole wheat, since whole wheat is the predominant grain in the U.S. For example, since whole wheat contains 11 grams of dietary fiber per 100 grams, the eligible amount of dietary fiber required for a food to bear the whole grain claim could be determined by the formula: 11 grams x 51% x RACC/100 (8).

Currently, the FDA has not defined any claims concerning the grain content of foods. However, they recommend looking at the food label and ingredient list to determine if a product consists of whole grains. Products labeled with "100 % whole grain" should only contain whole grains that the agency considers to be whole grain. The FDA also acknowledges that there are no standards of identity for products made from whole grains, however there are standards of identity for whole wheat bread, rolls, buns, and whole-wheat macaroni products. This means that for bread, rolls and buns, the dough is made from whole-wheat flour, brominated whole-wheat flour, or a combination of these and no other type of flour is used. In terms of label statements about whole grains that are currently permitted on food products, the FDA allows manufacturers to make factual statements. These statements include labels such as “10 grams of whole grains”, “1/2 ounce of whole grains,” and “100% whole grain oatmeal”, and do not include statements which imply that a particular level of the ingredient are “high” or “excellent” (9).

In order to help consumers choose whole-grain products, the FDA has allowed the food industry to stamp products with the grams of whole grain per serving. The “basic” stamp is allowed on food products that provide 8 grams or more whole grain per serving (contain at least half a serving of whole-grain ingredients). These products may contain some extra bran, germ, or refined flour. The “100%” stamp is allowed on foods that provide at least 16 grams or more of whole grain per serving. For these products, all of the grain in the ingredient list is from whole grain. The United States Department of Agriculture (USDA) recommends consumers read the ingredient list of the food in question and chose foods that have whole grain listed first in the ingredient list. Many foods contain whole and refined grains. If other non-whole grains are listed in the ingredient list, it may be assumed that the product is a partial whole-grain product. Determining if a product is truly whole grain is becoming difficult because a growing number of partial whole grain products are entering the marketplace. If a product lists multiple whole-grain ingredients, they should appear near the beginning of the ingredient list to ensure that they make up most of the product. Jonnalagadda et al. (10) recommend only counting products whole grain if every grain listed in the ingredient label is whole. Due to the lack of detailed information about the quantity of whole- and non-whole grain ingredients in partial whole grain products, this is a simple option to determine if a product is 100% whole grain. Harris et al. (11) add that terms such as “whole wheat” or “multi-grain” are not regulated by the FDA and can add to the confusion when choosing whole grain products. Lastly, the Dietary Guidelines for Americans 2010 recommend choosing whole-grain products based on fiber content (12).

Whole Grain Composition

As mentioned before, a whole grain is made up of three portions: the outer bran layer, the inner germ and endosperm. Whole grains are a good source of B vitamins, minerals, basic amino acids, and phytochemicals (2). There is a great deal of variability among various whole grains in their content of macronutrients, micronutrients, and phytochemicals. The major nutrients and phytochemicals found in whole grains are as follows: protein, fat, carbohydrate, dietary fiber, calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, thiamin, riboflavin, vitamin B-6, niacin, folate, vitamin A, vitamin E, vitamin K, selenium, beta-carotene, lignan, isoflavonoids, phenolic acids, and phenolic lipids (6). The most commonly consumed whole grain in the U.S. is whole wheat with composition as follows: 14% bran, 2.5% germ, 13% total dietary fiber (11.5% insoluble dietary fiber, 1.1% soluble dietary fiber), 14% protein, 2.7% fat, 70% starch and sugar, 1.8% total minerals, 7.5 milligrams/gram vitamin B-6, 32.8 micrograms/grams vitamin E, and 2.9 milligrams/gram phytate phosphorous (13).

More specifically, the bran is a multi-layered shell made up of nondigestible, mostly insoluble, low fermentable carbohydrates such as cellulose and hemicellulose (10). It also contains phenolic compounds along with vitamins and minerals (10). The bran functions as an outside shield to protect the inside germ and endosperm from damage, sunlight, pests, water, and disease (10). The starchy germ and endosperm contain viscous soluble fibers (found mostly in oats and barley), fermentable oligosaccharides, resistant starch, lignans, vitamins, minerals, polyphenols, oils, and many other phytonutrients (13). The endosperm is the largest portion of the grain, rich with carbohydrates, protein, vitamins, and minerals to serve as a nutrient supply for the germ and the rest of the plant (13). The germ is the embryo of the grain and is where a new plant sprouts (10). It contains vitamins, some protein, minerals and fat (10). The aforementioned components are thought to contribute to the healthfulness of whole grains.

Whole grains are considered a functional food; this is because their components may provide health benefits. Functional foods are made up of substances called phytochemicals, which may promote good health but are not considered nutrients because they are not essential for life. The beneficial elements of a whole grain are concentrated mostly in the bran and germ portion of the grain (14). Whole grains are also made up of essential macro- and micronutrients. Adom et al. (15) explain that most of the beneficial phytochemicals and nutrients are found in the bran as well as the endosperm of the whole grain. Okarter et al. (16) list phenolics, carotenoids, vitamin E, dietary fiber, and beta-glucan as the most important groups of whole grain phytochemicals. The most studied phytochemical, known as phenolic acids, provide chemical defense for the grain against pathogens, parasites, and predators. Specifically, in wheat kernels, a phenolic acid known as ferulic acid provides protection by employing physical and chemical barriers through cross-linking with carbohydrates, antioxidant activity, and astringency that prevents consumption by predators. When a whole grain is ingested, the phenolic acids function as antioxidants by donating hydrogen atoms to free radicals. Of all the grains tested, corn is reported to have the highest phenolic acid content, followed by wheat, oats, and rice (16).

Another phenolic acid to note is alk(en)ylresorcinol, generally found in the bran layer of the grain. This compound is prominently found in barley, rye, and wheat. It has never been detected in corn, millet, oats, rice or sorghum. Alkylresorcinol is often used as a biomarker for whole grain intake assessment (7).

Found in the germ and bran fraction of the grain is another type of phytochemical, carotenoids, these include lutein, zaexanthin, beta-cryptoxanthin, beta-carotene, and alpha-carotene. These are most commonly known for giving the grain pigmentation and function in reproduction and protection while also providing some antioxidant activity (10).

B vitamins such as thiamin, riboflavin, and niacin found in whole grain play a key role in metabolism. Vitamin E and its derivatives are mostly found in the germ fraction of whole grain and functions to maintain the integrity of the cellular membranes and also has a protective effect because of its antioxidant activity (7). Vitamin E is found in whole grains such as barley, bulgur, corn, rye, wheat, and wild rice (6). Minerals found in whole grain include iron, magnesium, and selenium (10).

Another biologically important component of whole grain is fermentable carbohydrates. The content of fermentable carbohydrates varies depending on the type of grain. Types found in whole grain include dietary fiber, resistant starch, and oligosaccharides. These are considered fermentable carbohydrates because they are undigested and once they reach the colon they can be fermented by intestinal microflora producing short-chain fatty acids and gases. Short-chain fatty acids such as butyrate, is a preferred fuel for the cells of the colonic mucosa (2). Slavin et al. (13) state that oats, rye, and barley only contain about one-third soluble fiber while two-thirds is made up of insoluble fiber. Furthermore, wheat is known to be lower in soluble fiber compared to other grains and rice contains close to no soluble fiber. The insoluble portion of the undigested carbohydrate helps to increase fecal weight and speed intestinal transit time. These functions have been known to provide health benefits (17). Insoluble fiber is mostly concentrated in wheat bran and corn bran (14).

According to the AACC, dietary fiber is the edible parts of plants or analogous carbohydrates that is resistant to digestion and absorption in the human small intestine and is completely or partially fermented in the large intestine (5). Some components of dietary fiber that are found in whole grain include lignin, hemicellulose, cellulose, and beta-glucan. Lignin is a compound found in the cell walls of plants and forms complexes with cellulose and hemicellulose, which is extremely resistant to degradation (18). Beta-glucan is a dietary fiber mostly found in oat bran, oatmeal, oat flour, barley, and rye (14).

Cummings et al. (5), state that another fiber found in whole grain known as resistant starch is the sum of starch and products of starch digestion that are not absorbed in the small bowel. Resistant starch I is mostly present in whole grains and is a physically inaccessible starch such as partly milled grains. Thus, resistant starch I passes to the colon where it can be fermented by gut bacteria to produce short chain fatty acids and increase stool output (5).

Oligosaccharides, such as fructooilgosaccharides, are another type of fermentable carbohydrate found in whole grains that consist of linked monosaccharide units (5).

Slavin et al. (17), describe oligosaccharides as having similar affects as soluble fibers in the human gut in which portions that are undigested can serve as fuel for intestinal microflora. Cummings et al. (5), goes on to explain that oligosaccharides are a source of short chain fatty acids, which alter the balance of microflora. Van Loo (19) et al. estimates that about 78% of the North American intake of oligosaccharides comes from wheat.

Compounds called antinutrients are also found in whole grains. They may contribute to the overall protective effect that whole grains are known for by suppressing oxidative damage, thereby protecting the intestinal epithelium. A reduced risk of certain cancers has also been reported with the consumption of antinutrients found in whole grains (10). Some antinutrients include, phytic acid, hemagglutinins, tannins, and digestive enzymes (protease and amylase). They also function by interfering with the absorption of nutrients such as minerals. (2).

Whole Grain Processing

Processing of grains is common in developed countries and is often necessary in order to extend the shelf life of the food. Processing techniques include: milling, heat processing, and extrusion (puffing). These processing conditions prepare the grain for digestion and absorption in the body. Some research suggests that processing may positively affect the nutritional quality of the grain by preserving the nutritive value of the grain. However, the milling process does remove many of the beneficial compounds found in the outer layers of the grain. Slavin et al. (1) describe the processing of grains and its effect on nutrition. The author reports that very few consumers actually eat the intact kernels of grain. This is helpful because the nutrients and phytochemicals in intact whole grain would not be as well digested and absorbed in the body as ground grains (1).

Harris et al. (11) explain that processing grains may provide health benefits. More specifically, processing can improve the palatability and digestibility of whole grains and even increase antioxidant capacity. More specifically, a process known as extrusion which uses high temperature, pressure, and shear to “puff” the cereal may cause the interconversion between insoluble and viscous fiber (11). During milling, when the bran and germ are separated from the endosperm (to be ground into flour) the nutritional quality of the grain may be decreased but the loss of phytates and fiber increases the bioavailability of certain vitamins and minerals (1). Another processing technique known as grinding also increases bioavailability of all nutrients in the grain. In fact, tightly bound phytochemicals may be released from the food matrix during processing (2). Thermal processing such as cooking the whole grain is known to release antioxidants from the bran into the endosperm, increase bioavailability of phytochemicals, and form resistant starch. The author concludes with the idea that understanding the synergistic and processing effects of whole grains on health are needed (11).

Refined Grain Composition

The majority of the U.S. population has only been consuming refined grains for the past 140 years (2). In 1873, a roller mill was introduced that could efficiently separate the bran and germ from the endosperm. Separating the germ was often necessary because it is high in unsaturated fat and could affect the shelf life of the food (1). The germ portion, rich with unsaturated fat, may become rancid over time in whole grains and is removed in refined grains and so contributes to its longer shelf life. Due to consumer demand of refined grain products, the use of the machine became widespread and consequently a dramatic decline in whole-grain consumption was observed between 1870 and 1970 (2).

A refined grain contains only the endosperm, as the refining process removes the germ and bran. The endosperm is then ground and made into flour, which can then be incorporated into foods. Consequently a majority of the fiber, vitamins, minerals, and phytochemicals are lost. Whole grains contain about 80 % more fiber than refined grains and also have a higher content of antioxidant activity compared to refined grains. The refining process removes more of the insoluble fiber than soluble, however refined grains are low in total dietary fiber (10). Because refined grain mostly consists of endosperm, it has higher starch content than whole grains (13). Most refined grains are enriched in order to replace the vitamins and other nutrients lost in the refining process (10).

Whole Grain and Health

The connection of whole grain consumption and health benefits were suggested long ago. During the 4th century BC, the father of medicine, Hippocrates acknowledged the health benefits of whole grain bread. Later on, whole grains were recommended to prevent constipation by physicians and scientists (2). Today, whole grains have been linked to reducing the risk of cardiovascular disease (CVD), cancer, diabetes, and obesity. The additive and synergistic effects of all nutrients and phytochemicals found in whole grain are thought to be responsible for the health benefits associated with whole grain consumption (10). The basis of these findings is mainly derived from observational studies (18).

Evidence based recommendations regarding whole grain intake and health is lacking and sometimes contradictory (4). Epidemiological studies have shown that a diet rich in whole grains is associated with reducing the risk of chronic diseases such as certain forms of cancer, CVD, and diabetes (20, 21, 22, 23). Whole grain consumption has also been related to weight maintenance, blood glucose levels, and all-cause mortality (22). Therefore, it is assumed that whole grains are important for a healthy lifestyle and it is recommended that adequate amounts be consumed (24).

A problem that comes along with observational data is that causality cannot be assigned. Therefore, it is unknown whether a diet rich in whole grains is protective or if it is simply a marker of a healthy lifestyle. Also, different grains are made up of different compositions of macronutrients, micronutrients, and phytochemicals, therefore each may provide different effects that may be involved in decreasing the risk of certain diseases. For example, wheat is composed of more insoluble fiber while oats are known to be a good source of beta glucan and soluble fiber. Intervention studies are needed to provide evidence of the health benefits of whole grain consumption (11).

Whole Grain and Cardiovascular Disease

Okarter et al. (7), describe the health benefits of whole grain phytochemicals in which the most recent observational studies that are associated with health benefits and whole grains are reviewed. One of the first studies reviewed, by Jacobs et al. (25), shows an inverse association between whole grain intake and ischemic (reduced blood supply) heart disease from the Iowa Women’s Health Study, after adjusting for total dietary fiber intake. A few years later, Pietinen et al. (26), report that when whole grain products, mostly rye, were given to a study population, an inverse association was seen with coronary death. The next year, Liu et al. (27) reported results from the Nurse’s Health Study. The researcher concluded that there was a strong inverse association between whole grain intake and the risk of coronary heart disease for women in the highest quintile (3 servings per day) for whole grain consumption when compared to the lowest. Interestingly, this association held true for both nonfatal coronary heart disease and fatal coronary heart disease. Consequently, increased consumption of whole grains may protect against CHD.

That same year, Fraser et al. (20) looked at whole grain preference and the incidence of ischemic heart disease. The author reports that the preference for whole-grain bread instead of preference for white bread was associated with reduced risk of ischemic heart disease. In 2000, Anderson et al. (28) reported findings on a meta-analysis of studies that investigated the relationship between whole grain consumption and the risk of coronary heart disease (CHD). After an adjustment for primary (demographic data) and secondary (total energy intake, body mass index, smoking, and blood pressure) risk factors, four of the twelve studies reviewed showed a significant inverse association between whole grain and whole wheat bread intake and risk of CHD. The results indicate a significant reduction of risk of 26% on average for those consuming a regular intake of whole grain food (3 servings per day). Lastly, results from a case-control study performed in Norway, reported by Lockheart et al., (29) showed an association between dietary patterns and the risk of first myocardial infarction. Once the researcher adjusted for family history of heart disease, smoking, energy intake, and other possible confounding factors, whole grain breakfast cereals, not whole grain bread, were inversely associated with risk of first myocardial infarction when comparing the group with the highest level of whole grain intake to the group with the lowest. The epidemiological data shows that the consumption of whole grains is associated with reduced risk of CVD.

The previously described observational studies establish an association between CVD and whole grain intake. In addition, intervention studies are needed to prove causality. In 2010 Tighe et al. (30) performed an intervention study to determine the effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons. The study assessed the effects of consuming three daily portions of whole-grain foods (wheat or a mixture of wheat and oats) on markers (blood pressure, cholesterol and triglyceride measurements) of CVD in relatively high-risk individuals. A total of 206 participants included in the study, started with a run-in period consuming refined grains. Then were randomized into a control group (refined diet), wheat diet, or wheat and oats diet for 12 weeks. The researcher found, for the first time, that daily consumption of three portions of whole grain foods significantly decreased systolic blood pressure and pulse pressure in middle-aged, otherwise healthy, overweight men and women (30).

Contrary to the findings described above, a randomized, controlled, dietary intervention found that the markers of cardiovascular risk are not changed by increased whole-grain intake. In 2010, Brownlee et al. (31) reported findings of the WHOLEheart study, which included 316 participants between the ages of 18 and 65 years old. The participants were randomly assigned to three groups: control, intervention 1 (60 g WG/day for 16 weeks), and intervention 2 (60 g WG/day for 8 weeks followed by 120 g WG/day for 8 weeks). The researcher found no significant difference in any markers of CVD between groups (31). Compared to the intervention trial described previously, which used 6 blood pressure measurements on average, the WHOLEheart study blood pressure results were averaged from only two consecutive measurements. The differences in findings illustrate the limitations of randomized, controlled, intervention trials among humans although clinical trials hold more credibility than observational studies (30).

Whole Grain and Diabetes

Modifications in lifestyle, such as changing eating habits, are major factors in the prevention and treatment of diabetes. In order to assess the effects of consuming whole grains on the risk of type II diabetes, numerous epidemiological studies have been performed. Starting in 2000, Liu et al. (32) investigated the relationship between whole grain and refined grain intake and the risk of type II diabetes. A positive association between refined grain intake and risk of type II diabetes was found when the highest quintile of refined grain intake was compared to the lowest quintile after adjusting for age and energy intake. When looking at whole grain intake and the risk of type II diabetes, a significant inverse association was found. According to the author, the results provide supportive evidence that consumption of whole grains is associated with reduced risk of developing type II diabetes (33).

Another study performed in 2000, Meyer et al. (34) also looked at the consumption of whole grains and type II diabetes. Whole grain consumption was found to be inversely associated with the risk of type II diabetes when comparing the highest quintile of whole grain intake to the lowest. A study performed in 2002, using data from the Health Professionals Follow-up Study, found that the subjects who consumed diets high in refined grain remained positively associated with risk of type II diabetes (35). Later in 2002, Fung et al. (36) also used data from the Health Professionals Follow-up Study to investigate the association between whole grain intake and risk of type II diabetes. Whole grain consumption was inversely associated with the risk of type II diabetes after adjusting for fruit and vegetable intake and other confounding factors. In 2003, Monteonen et al. (37) looked at whole grain intake and the risk of type II diabetes using data from the Finnish Mobile Clinic Health Examination Survey. When compared to the highest quartile of whole grain consumption to the lowest, and after adjusting for fruit, berry, and vegetable consumption and other confounding factors, whole grain consumption was inversely associated with the risk of type II diabetes. A more specific study by de Munter et al. (38), in 2007, investigated the association between whole grain, bran, and germ intake using data from the first and second trials of the Nurse's Health Study. After adjusting for confounding factors such as physical activity and energy intake, the author found that whole grain and bran intake (2-serving per day increase) was inversely associated with risk of type II diabetes. This resulted in a 21% decrease in risk of type 2 diabetes. According to the observational data described here, an association between whole grain intake and type II diabetes has been established.

Whole Grain and Obesity

Epidemiological studies illustrate that consuming whole grains is associated with reduced risk of obesity and weight gain (10). To date, 14 cross-sectional studies have been performed in order to investigate the relationship between whole grain intake and weight management. All 14 of the studies found that higher intake of whole grains (a daily intake of 3 servings) are associated with lower BMI in adults (39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51). In 2003, Liu et al. (41) investigated the association between whole grain consumption, body weight, and long-term body weight changes using results from the Nurse's Health Study. The author found that women who consumed larger amounts of whole grain consistently weighed less than women who consumed smaller amounts of whole grain.

Using data from the Physician's Health Study, Bazzano et al. (43) reported the association between whole and refined grain breakfast cereal consumption and weight gain in men. The results demonstrate that whole grain breakfast cereal consumption was inversely associated with risk of having a BMI greater than 25 and weight gain of more than 10 kg, 8 years after initial subject evaluation after adjusting for baseline BMI, physical activity, age, and other confounding factors. Few randomized, controlled, trials have been conducted to see the effect of whole grains on weight management. Of the few that have been done, whole grains did not facilitate weight loss. Nevertheless, more intervention studies are needed to learn the effects of a diet rich in whole grains on weight (11).

A study to specifically test the hypothesis of whether whole grain consumption is associated with lower body mass index among adolescents was performed by Steffen at el (40). The study involved 285 adolescents between the ages of 13 and 15 years old. After adjusting for age, gender, race, Tanner stage, and energy intake, the mean BMI for adolescents consuming less than ½ serving of whole grain foods per day was 23.6, while the mean BMI for adolescents consuming more than 1 ½ servings per day was 21.9. The author concludes that whole grain intake is associated with lower BMI in adolescents, particularly for the heaviest children. A higher intake of whole grain foods was also associated with a healthier dietary profile, consisting of greater intakes of fruit and vegetables, fiber, iron, zinc, calcium, folate, and vitamin E. Additionally, the results show that initiating diets rich in whole grains during the adolescent years would be beneficial for weight management (40).

Whole Grain and Cancer

Many epidemiological studies have been performed in order to determine if a relationship between whole grain intake and relative risk of certain cancers can be established. The 4th most common type of cancer, colorectal cancer, contributes to 53% of all gastrointestinal cancers (10). From 1995-2007, four studies looked at whole grain consumption and colorectal cancer. In 1995, Jacobs et al. (21) reported an inverse association between whole grain consumption and risk of colorectal cancer. Four years later, Pietnen et al. (26) used data from the Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study and reported no association between colorectal cancer and intake of various rye products. These findings do not agree with the assumption that increased intake of whole grains decreases the risk of cancer, specifically colorectal cancer. In 2005, Larsson et al. (52) used data from the Swedish Mammography Cohort in order to report the association between whole grain intake and incidence of colon cancer. The author found that whole grain consumption was inversely associated with risk of colon cancer after adjusting for red meat, fruit, and vegetable intake and other possible confounding factors. In agreement with Larsson et al. (52), in 2008, Schatzkin et al. (53) also found an inverse association between whole grain consumption and risk of colorectal cancer. Interestingly, the association was stronger for men than for women and the reduced risk of site-specific tumors was strongest for the rectum (53).

Other cancers have also been of interest. Nicodemus et al. (54) investigated whole grains and breast cancer. Women in the lowest quintile of whole grain intake had a lower risk of breast cancer incidence compared to women in the highest quintile. Other studies report that there is an unclear association between dietary fiber and risk of death from breast cancer (55). Currently, more research is needed to further understand the relationship of whole grain and breast cancer.

Kasum et al. (56), using data from the Iowa Women's Health Study, studied endometrial cancer and whole grain intake. Findings suggest that for women who have never received hormone replacement therapy, whole grain consumption was inversely associated with risk of endometrial cancer. However, no association was found between whole grain consumption and risk of endometrial cancer for the rest of the women in the study when confounding factors were adjusted for. Chan et al. (57) found a strong inverse association between the risk of pancreatic cancer and the highest quartile of whole grain consumption to the lowest. Data was collected from a large population-based case-control study on pancreatic cancer and adjusted for history of smoking, red meat, fruit, and vegetable intake (57).

Currently, researchers agree that it is not possible to determine exactly which specific components in whole grain are responsible for reduced risk of disease. It is suggested by Frolich et al. (4) that there is most likely a combination of different components providing such an effect. The author goes on to explain that because whole grains are made up of individual components correlated in different ways to each other, it is near impossible to separate their effects (4). Jonnalagadda et al. (10) describe this point even further, stating that health benefits associated with whole grains may be from the additive and synergistic effects of these bioactive phytochemicals.

Epidemiological data has formed a foundation for the Dietary Guidelines 2010 to recommend that Americans consume at least half of their grains as whole grains. In 1999, the whole grain health claim was permitted by the FDA, which reads, "Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers" (8). Furthermore, the FDA claims that there is a body of scientific research that provides compelling evidence that whole-grain consumption among the US population would have a marked impact on public health by reducing risk for some types of cancer and CHD (8).

Whole Grain Recommendations

The United States Department of Agriculture reports that Americans are getting enough total grains, however most are coming from refined grains. For the past 20 years, the USDA has suggested that whole grains are an essential factor for a healthful diet (58). The National Research Council's Diet and Health: Implications for Reducing Chronic Disease Risk also recommends the consumption of whole grains (59).

A food guide for the general population was created by the USDA called ChooseMyPlate to provide nutrient adequacy information for individuals, depending on their age, sex, and activity level (60). According to ChooseMyPlate, at least half of the recommended total grain intake should be from 100% whole grains. For many, this would amount to consuming 3-ounce equivalents or more of 100% whole grains each day. A slice of bread is an example of 1-ounce equivalent. The amount of grains needed per day depends on age, sex, and level of physical activity. More specifically they suggest that adolescent males require between 6- to 7-ounce equivalents of grains per day with at least half being whole and adolescent females require 5- to 6-ounce equivalents of grains per day with at least half being whole. Health organizations along with the U.S. government recommend people to consume more whole grains in accordance with research that has shown a relationship between positive health outcomes and whole grain consumption. The key consumer message the USDA is trying to get across is to make at least half of the grain intake whole grain (61).

To determine if people are consuming the recommended amount of whole grains per day, diet information from large epidemiological studies is analyzed. According to published data, adolescents seem to be an age group that is consuming well below the recommended amount of whole grains per day (62, 63, 64). Meeting the recommendations during the adolescent years is very important for growth and health outcomes they may affect adulthood and beyond.

Adolescent Whole Grain Consumption

In order to determine the average grain intake for adolescents, Harnack et al. (64) examined data from the 1994-1996 Continuing Survey of Food Intakes by individuals. From the survey, the intake and food sources of whole grains among adolescents aged 2 to 18 years old (n=4,802) was determined. The participants provided 2 days of dietary recall data administered by using the Continuing Survey of Food Intakes by Individuals (CSFII) method. The CSFII method consists of two nonconsecutive days of dietary data, collected using a one-day, interview administered, recall. The major sources of whole grains indentified were ready-to-eat cereals, corn and other chips, and yeast breads. The average whole grain intake for the adolescents sampled was 1.0 serving per day according to serving size as defined by the Food Guide Pyramid. It is noted that an aggressive public health intervention targeted to children and adolescents are required to implement whole grain recommendations. In conclusion, the reported low levels of whole grain consumption in adolescents demonstrate that the recommended amount is not being met (64).

Trends of whole grain consumption in adolescents have also been studied. In 2010, Burgess-Champoux et al. (63) reported 5 years (1999-2004) of longitudinal and secular trends in adolescent whole-grain consumption. Data was collected from 996 males and 1222 females who were part of Project EAT (Eating Among Teens). In 1999, only 11% of adolescent males and 13% of adolescent females consumed more than one serving of whole grains per day. No significant changes in whole-grain consumption for either sex were seen over the 5 years. Overall, the reported average daily intake of whole grains was below the recommended amount, for both sexes. Some potential barriers were reported as reason for the low amount of whole grains consumed, such as taste, appearance, cost, texture, knowledge of health benefits, and ability to identify whole grain products at the grocery store (66). The findings suggest that efforts should be made to help adolescents increase their whole grain intake. The researchers advocate the development of an intervention in order to improve the whole-grain intake among adolescents to the recommended amount (63).

In 2010, Larson et al. (62) reported data from Project EAT-II and also suggest that an intervention to provide adolescents with whole grains is needed in order to increase whole grain consumption to the recommended amount. It was found that mean daily intake of whole grains was lower than recommended, among adolescents. The author suggests that young people need to be provided with opportunities to taste a variety of whole grains in order to enhance taste preferences and awareness of whole grain products (62).

Low intake of whole grain was confirmed in another study, which associated improved diet quality and nutrient intake in children and adolescents consuming whole grains. O’Neil et al. (65) used data from the National Health and Nutrition Examination Survey for 1999-2004, and found that adolescents 6-12 years old consumed an average of 0.59 servings of whole grain per day and adolescents 13-18 years old consumed 0.63 servings of whole grain per day. The data demonstrates that overall consumption of whole grain in adolescents is low. These intakes are even less than previously reported averages of whole grain intake among adolescents. Although whole grain intake was low among adolescents, an increased consumption of whole grain was associated with improved diet quality and nutrient intake. The improved diet quality may have the potential to improve even more if daily recommendations of whole grain intake were met in adolescents (65).

According to the Whole Grains Council, in 2000, there were 164 whole grain product launches. Fast-forward to 10 years later, and 3,272 new products have been launched. Additionally, the whole grain stamp is now on over 5,300 different products across 22 countries. The council also provides data on the rise in overall whole grain consumption over the years. Apparently, from 2005 to 2008, consumption rose 20% after remaining steady from 1998 to 2005 and in 2008, 60% of Americans consumed at least one whole grain product during a typical two-week period. Moreover, 18 to 34 year olds increased their consumption the most from 2005 to 2008, rising 38%. In conclusion, the group goes on to explain that Americans are still not eating the recommended amount, with only 11% eating their grains as whole grains (67).

Observational data has been assessed in order to determine the current amount of whole grains being consumed by adolescents (64, 62, 63, 65). Overall, adolescents are consuming below the USDA recommended amount. A study to reveal the impact of providing grain foods (e.g. cereals, breads, and pastas) to adolescents has never been conducted and is needed to determine if adolescents will consume the recommended amount of whole grains if it is provided to them.

Perceptions Regarding Whole-Grain Foods

The USDA has been recommending the consumption of whole grain foods for many years, however overall, adolescents are not incorporating these foods into their diets at the recommended amount. Including whole grains in the diet and establishing healthful eating habits at a young age may carry over to adulthood, thereby reducing the risk of chronic diseases. Burgess-Champoux et al. (66) report some common barriers exist that may be contributing to the low intakes of whole grain foods among adolescents. Some of the potential barriers are taste, appearance, cost, texture, consumer knowledge of health benefits, and ability to identify whole-grain products. Perhaps learning about the perceptions of young people regarding whole grain foods could eliminate some of these barriers and increase overall consumption of whole grain.

In 2006, a study was performed to explore perceptions of whole-grain foods and factors influencing intake of children (n=40). Focus group interviews were arranged for children in grades ranging from K-6th grade. During the interview, a series of discussion questions were asked while a variety of whole-grain and non-whole grain bread and cereal products were shown. Most of the participants (70%) indicated they always or often ate school lunch. Along with questions pertaining to individual factors such as physical and socioenvironmental factors including the availability of whole-grain foods, sensory characteristic questions were also asked to get a sense of the children’s taste rating of whole grain foods. Most children indicated that they wanted to eat the whole-grain cereal and a majority liked the appearance, taste, and texture of the product. This study provides insight into the perceptions and views of whole grains according to a small group of children, however more research is still needed to optimize ways to increase whole grain consumption to the recommended amount (66). Findings from Project EAT (Eating Among Teens) report a conceptual model of factors influencing whole-grain intake in adolescents, which include sociodemographic factors (race/ethnicity), socioenvironmental factors (support for healthy eating), behavioral factors (meal frequency), and personal factors (taste preference) (62). Still, it is reported that little is known about the barriers to whole grain consumption in adolescents.

Dietary Assessment

Dietary assessment has increased in popularity since the link between diet and disease was established (68). More importantly, the accuracy of diet assessment has been an ongoing area of research since diet reporting can be so variable. This is needed in order to evaluate the diet precisely and validate findings. Dietary assessment is also a very valuable measure for clinical nutrition studies such as interventions, implementing new dietary guidelines or to see if guidelines are being followed. Measuring the diet of a specific population, to determine if they are meeting recommendations, requires some form of dietary assessment. The United States Department of Agriculture's Dietary Guidelines for Americans 2010 are based on past diet assessments of the US population, among other things. Diet assessment strategies are very important for nutrition assessment, education and research.

Dietary Assessment Methodology

The Nutrition in the Prevention and Treatment of Disease by the National Cancer Institute describes some commonly used methods for individual level diet assessment. These include dietary record, 24-hour diet recall, food frequency, brief dietary assessment instruments, diet history and blended instruments. These methods are all used to assess diet in a research setting (69).

Diet Records

A diet record is an approach in which the respondent records food and beverages and the amounts consumed over one or more days. It is suggested that foods and beverages be recorded shortly after they are consumed. Recording periods should last no more than 3 or 4 days in order to decrease the risk of respondent fatigue. Intake has been known to be recorded using journals, dictation machines, computers, and self-recording scales. In order for the respondent to accurately record their intake they must be trained at a level to enable them to report measurements of food and beverages (69). Using food records as a means to measure dietary intake has some advantages and disadvantages.

Advantages for using food records include quantification of intake, the possibility of enhancing self-monitoring for weight control or other behavior change, and not requiring the recall of foods eaten. However, some disadvantages include high investigator cost, high respondent burden, extensive respondent training and motivation, the need of many days to capture an individual's usual intake, affecting eating behavior (such as changing diet for ease of reporting), underreporting intake, and reports of intake decreasing with time (69).

According to the authors, dietary records may be considered an "imperfect gold standard" for dietary intake assessment (69). This is because many previous studies found that intake was underreported when using diet records compared to energy expenditure as measured by doubly labeled water (70, 71, 72, 73, 74, 75). However, it is still known as the “gold standard” when it comes to assessing diets for longer than 24 hours but no more than 3 to 4 consecutive days. (68).

24-Hour Diet Recall

A 24-hour diet recall is a method used in which the respondent is required to give information about their dietary intake during a 24-hour period, typically the day prior. It has also been described as a “snap shot” of what a person has eaten. A 24-hour diet recall can be gathered by an in-person interview, over the telephone, using a computerized interview, or using a pencil and paper form. In order for a 24-hour diet recall to be most accurate, it is crucial that the interviewer be well trained in food measurements and interview skills. The interviewer uses specific questions and statements in order to probe the respondent for details concerning consumption. This has been known to help the respondent recall what was consumed in the past 24-hours. Interviewers are usually dietitians or well-trained interviewers that have an extensive knowledge of foods and beverages in the marketplace as well as measurements. Currently, the most validated 24-hour diet recall used is one in which a multiple pass method is implemented (69).

A 24-hour diet recall instrument has been standardized by the United States Department of Agriculture called the Automated Multiple Pass Method. It is used in the What We Eat in America survey, a nationally representative dietary survey in the United States. It is termed “multiple pass” because the respondent goes through the process of recall and questions numerous times. The methods consists of five passes: (i) an initial “quick list”, (ii) a forgotten foods list of 9 food categories commonly omitted in a 24-hour recall report (iii) time and occasion, (iv) a detail pass with probing questions about portion size, eating occasions and times and lastly, (v) final review. For each recall the description of the food, amount of food consumed, additions to the food (cream in coffee), time eaten, name of eating occasion, where obtained, and whether it was eaten at home or not is recorded. This method provides many advantages for the researcher (69).

Advantages for using the 24-hour diet recall method for dietary assessment includes, being able to quantify intake, being appropriate for most populations, having relatively low respondent burden, and not affecting eating behavior. There are however some disadvantages, to include high investigator cost, many days needed to capture the individual's usual intake, and underreporting or forgetting food consumed (69). It has been reported that 24-hour diet recalls are most useful in population based studies. Depending on the number of days needed for dietary analysis, a 24-hour diet recall can be used in order to determine dietary patterns within a sample (68).

Food Frequency

Another approach for dietary assessment described in the National Cancer Institute publication is called food frequency (69). Food frequency questionnaires (FFQ) are administered in order to sum the overall nutrient intake of an individual over the past week, month or even year. Questionnaires can be developed which target a specific population, such as the diet of Native American Indians. The main focus in the food frequency approach is to get a general idea of the usual foods consumed. The respondent reports their usual consumption based from a list of foods. Generally, portion size is collected while more specific characteristics such as cooking methods or combination of foods in a meal are given in little detail. A typical FFQ consists of 100 individual items with additional portion size questions and may take up to 60 minutes to complete (69).

Some advantages for using an FFQ to collect diet information is that the usual individual intake is asked, information about total diet is obtained, there are low investigator costs, and it does not affect eating behaviors. Moreover, some disadvantages include not being quantifiably precise, being a difficult cognitive task for the respondent and intake is often misreported (69).

Brief Dietary Assessment Instruments

Brief dietary assessments are used in order to obtain crude information about the diet using a short screener. The screener may focus on one food group or incorporate multiple dimensions. For example, a fruit, vegetable, and dietary fiber screener may be used in order to determine a study participant's fiber consumption before starting a study. Generally, 15 to 30 foods are used to estimate nutrient status. This enables large groups of people to be grouped, such as those with low fiber intake. This can be helpful for research criteria, to track changes in the diet or for direction in nutrition education (69).

Using brief instruments in order to assess dietary intake has its advantages. These include, low investigator costs, low respondent burden, not affecting eating behavior and asking about the usual intake. Some disadvantages of using a brief instrument include, not being a quantifiably precise method, being a difficult cognitive task, and intake often being misreported. The authors suggest that brief instruments be used to assess dietary intake for specific food groups and not as a means for capturing an overall evaluation of the diet (69).

Diet History

Originally the term "diet history" was thought by Burke and was defined as the collection of information not only about the frequency of different foods but also about the typical composition of meals. Today, however, the term is used loosely to describe many assessments. The authors describe diet history as being most beneficial in determining a person's usual food intake in which many details about characteristics of foods as usually consumed are assessed in addition to the frequency and amount of food intake (69).

The Burke diet history encompasses three parts: a detailed interview about usual pattern of eating (much like a 24-hour diet recall), a food list asking for amount and frequency usually eaten, and a 3-day diet record (69). These assessments are meant to ascertain the usual eating patterns for an extended period of time. Many other diet history methods have been formulated around the original Burke diet history. Some advantages of the method is that the usual intake is captured, information on the total diet is obtained, information about foods consumed by meal is obtained, there can be low investigator costs, and it does not affect eating behavior. Some disadvantages of the diet history method are that it is not quantifiably precise, it has difficult cognitive tasks, the intake is often misreported, and it can have a high degree of investigator burden (69).

Dietary Assessment in Intervention Studies

During an intervention study, dietary assessment is necessary to evaluate changes in the diet that may be due to the intervention. Additionally, to determine effects that may be from the intervention, diet assessment needs to be performed before and during the intervention study. Studies have been done to determine which method of dietary assessment is most often used during small and large-scale interventions. It has been reported that for small population studies, dietary records, multiple 24-hour diet recalls, and diet history questionnaires are most commonly used.

For larger scale studies, FFQs and brief instruments were used more frequently. Less precise measures of dietary assessment are generally used in larger studies because of resource constraint (69).

Dietary Assessment in Children and Adolescents

Assessing the diets of children and adolescents has known to be more challenging compared to adults. In particular, there are reports that adolescents may be more willing to report, however their interest in giving accurate reports may be a problem. Furthermore, Rockett et al. (76) explain that because a children's cognitive ability to record or remember their diets, as well as their limited knowledge of food and food preparation, there is an additional dimension of difficulty that must be addressed. Dietary assessment in children has been reviewed and many methods have been tested (68, 77, 76). It is suggested that dietary assessment in children and adolescents involve blended instruments and other creative ways of engagement and motivation in order to retrieve the most accurate measure of intake (69).

Rockett et al. (76), explains that the diets of young people have been evaluated using all of the previously mentioned methods and the development of these methods has been evolving since 1882. One of the first longitudinal studies, which looked at children's diets, began in 1973 using repeated 24-hour diet recalls. After the study began the investigators realized that measures of quality control, such as protocols for interviewing and training of the interviewers, the use of food models and pictures, school lunch analysis, and duplicate recalls were necessary in order to obtain the most accurate data (77). These measures of quality control are still implemented today in many studies involving dietary assessment.

Throughout the years, dietary assessment in children and adolescents has evolved from weighing food, to the development of the diet history, to standardized nutrition surveys and in the future, to computerized data collection programs. Rockett et al. (77) compare the validation of current methods and finds that dietary records, 24-hour recalls, and food frequency questionnaires all have their advantages and disadvantages, as described before. Therefore, it is to the discretion of the scientist to decide which method is best for a particular study. While diet records remain the "gold standard" for diet assessment, it is typically known to be a demanding method that requires a lot of writing and food measurements from the subject, this can be difficult in an adolescent population. FFQs and 24-hour diet recalls may be more adolescent-friendly because they both require less effort from the participant than diet records (76).

Rockett et al. (68) describe that the main considerations, when it comes to choosing which dietary assessment tool to use in adolescents are: motivation, cognitive ability and literacy level of the participant, the size and location of the study population, as well as the financial constraints and detail of nutrient/food analysis. Additionally, diet records should be used when a specific period of time needs to be accounted for (3-4 days), 24-hour diet recall should be used when an estimate of a populations' diet or a "snap shot" of the diet is needed, and FFQ should be used when an estimate of an individual's diet is needed over a long period of time (month-year) (68).

Issues in Dietary Assessment in Children and Adolescents

The adolescent years are a time of growth and development, including changes that are biological, social, and psychological. Because of these changes, dietary patterns and cognitive abilities may be different than during childhood or adulthood.

Understanding these patterns and abilities is the key to developing an appropriate diet assessment strategy in adolescents (78).

Livingstone et al. (79) describe the issues with dietary intake assessment of children and adolescents. More specifically, when it comes to the thought process of dietary reporting, some things that constrain a child's ability to self-report their food intake include their ability to estimate and indicate portion size and their knowledge of food. These problems have been indicated in children 12 years of age and younger. The author goes on to explain that by the ages of 8-10 years old children can reliably report their food intake, often as well as adults. When it comes to portion size estimations, adolescents as well as adults have been known to experience difficulty in reporting portion sizes and so one can conclude that the ability to estimate portion size is not age-related. Earlier work suggests that the best way to improve portion size estimation is to train the respondent (79). In a study by Weber et al. (80), a 45-minute training exercise in portion size estimation improved the accuracy of estimated food portions in children ages 9-10 years old.

Another issue related to dietary assessment in children is how they remember what they have eaten. Livingstone et al. (79) go on to describe the problem with how children remember, which can be one of the largest concerns when it comes to misreporting diet information. From previous work, the author describes that with all methods of diet assessment, there are problems with incorrect identification of foods and over- and under-reporting intake. Due to the lack of research, there is little known about the cognitive constraints on a child's ability to retain and retrieve dietary information (79). According to Baxter, et al. (81) in order to gain maximum recall from fourth grade children, non-directive prompts and cues have been shown to be helpful, such as those used in the multi-pass method of 24-hour diet recalls.

The main problems with dietary assessment accuracy are errors due to omission (failure to report items actually eaten) and intrusion (report of something not eaten at all). These problems are commonly seen in children and adolescent populations. Domel et al. (82) looked at self-reports of diet in order to describe how children remember what they have eaten. The author explains that learning how a child remembers what they have eaten may help in determining what kind of cue would be helpful in dietary assessment practices. The study involved 4th grade students, with an average age of 10 years old. Researchers observed the children while they consumed their school lunch and these results were compared with the student's report of what they consumed. The interviews were similar to the standard 24-hour diet recall method. During their diet interview the researcher asked the student, "How do you remember you ate ______?". This response was then used in order to compose retrieval response categories. The categories are as follows: visual imagery, usual practice (habit), behavior chaining (linking item to other items or activities), preference, lunch menu, smell-taste imagery, texture imagery, food-related events, food label, expected inquiry (from parent or interviewer), completion (eating all of the meal), and other (does not fit into other categories). The authors believe the development and use of cues during dietary assessments are needed, which may help the respondent remember what they have eaten (82).

A study was completed by Baxter et al. (83) to see the effects of shortening the retention interval of 24-hour dietary recalls in fourth-grade students. Over three school-years, 374 fourth grade students were observed eating two school meals and then interviewed using the 24-hour diet recall method with three interview times (prior 24 hour/morning, prior 24 hour/afternoon, and prior 24/evening). The researchers found that shortening the retention time of dietary recalls increases accuracy for reporting energy and macronutrients. In conclusion, when a researcher obtains the 24-hour diet recall is important for accuracy and needs to be considered when planning a study (83).

Validation of Diet Assessment Methods

Validation of dietary assessment is needed in order to determine the accuracy of the data obtained; this can be done using biomarkers or by comparing the tool in question to other diet assessment methods. The U.S. National Institutes of Health provides a dietary calibration/validation register for studies and their associated publications (84).

In 1990, energy intake from dietary assessments was validated using a biomarker called doubly labeled water (79). Doubly labeled water is an isotope-based method that measures the energy expenditure based on 2 isotopic labels in body water, 1 of hydrogen and 1 of oxygen. The doubly labeled water method is based on the theory that total daily carbon dioxide production can be measured from the differential elimination of water labeled with stable isotopes of hydrogen and oxygen. The labeled hydrogen will be eliminated as water, while the labeled oxygen will be eliminated as carbon dioxide. Next, the difference between the elimination rates of the labeled oxygen and hydrogen is measured and the carbon dioxide rate can be calculated. The rates at which the isotopes are excreted in the urine can be measured. This rate can then be converted into energy expenditure to be compared to energy intake recorded in dietary assessment methods. (85).

An article by Burrows et al. (86) reviews the validity of dietary assessment methods in children when compared with the method of doubly labeled water. Between 1973 and 2009, a total of 15 articles were reviewed that used the doubly labeled water technique to validate reported energy intake by any other dietary assessment method. The gathered studies were cross-sectional in design and reported on a total of 664 children. The majority of the participants were white children aged 4 to 11 years old. The most commonly used dietary assessment tools of the 15 studies reviewed were 24-hour multiple pass recalls (n=4 studies) and estimated foods records (n=5 studies). Other methods included diet history, weighed food records, and FFQ. In most of the studies, both the child and one or two of the parents/caregivers reported dietary intake (86).

Out of the 15 studies reviewed, all concluded some degree of misreporting. It was found that food records were underreported by 19% to 41% while over-reporting was associated with 24-hour recalls (7% to 11%), diet history (9% to 14%), and FFQ (2% to 59%). Overall, the authors suggest that the most accurate and convenient method for reporting energy intake in children aged 4 to 11 years old is the 24-hour multiple pass diet recall conducted over a 3-day period that includes weekdays and weekend days, using parents as the reporter. Furthermore, additional research is needed to further validate the conclusions. (86).

Validity of the Multi-Pass 24-hour Diet Recall

Since 1965, the USDA has been conducting nationwide surveys on the diets consumed by people in America using the 24-hour dietary recall method. Recently a group known as the ARS Food Surveys Research Group (FSRG) improved the method, which is now known as the multiple-pass method of 24-hour diet recall. This method uses the technique of guiding the participant through a 24-hour reference period of food intake more than one time, as previously described. Each pass probes for more detail and helps the subject recall information regarding their diet. Currently this method is being used for the dietary interview portion of the What We Eat in America survey to collect statistical data about the current dietary intake and health of people in the United States. The USDA's multiple pass method for collecting food intakes has been reviewed and validated by many published articles (87, 88, 89).

Revisions were made to the original method of 24-hour diet recall because of data showing that underreporting was common. The multiple-pass method hopes to improve accuracy by keeping respondents interested and engaged in the interview process, and to help them remember all foods they had consumed (90). The multiple-pass method is thought to help respondents recall more foods they consumed by asking about their intakes in different ways. Moshfegh et al. (88) reported that the new method was tested in a nationwide pilot study of 800 individuals and showed that the average calorie intakes and the number of foods reported were higher than data from 1996, using the original recall method (without multiple passes). This indicates that perhaps the multiple-pass method helps respondents remember and report more foods consumed than the original method (88).

Furthermore, the USDA multiple-pass method has also been evaluated by comparing reported energy intake with total energy expenditure using the doubly labeled water technique. Five hundred-twenty four participants, aged 30-69 years old, were included in a study in which doubly labeled water was dosed on the first day of the 2 week study period while three 24-hour diet recalls were collected using the multiple-pass method. Overall, the multiple-pass method accurately reported energy intakes, with approximately 78% of men and 74% of women classified as acceptable energy reporters (88). In conclusion, more research needs to be conducted to understand variability of the multiple-pass 24-hour at the individual level, however data shows this method to be accurate for obtaining dietary information among populations of adult men and women.

Validation of Diet Assessment Tools Developed Specifically for Adolescents

When it comes to gathering information about the diet, as previously described, adolescents have issues that need to be addressed. Measuring diet intake in adolescents is specifically complex because of issues with memory, portion size estimation, having variability in day-to-day intake and misreporting intake. Therefore, dietary assessments developed for adults need to be adapted for adolescents. Described here is some dietary assessment tools developed specifically for adolescents.

Rockett et al. (77) developed a Youth/Adolescent Food Frequency Questionnaire to be used to assess the diets of large populations of youths. The questionnaire is different than the standard FFQ for adults because the researchers refined the format and tried to make it relevant and simpler to complete. For instance, the Youth/Adolescent Food Frequency Questionnaire has a modified food list that reflects more of what adolescents consume. The validity of the Youth/Adolescent Food Frequency Questionnaire was tested with a sample of 261 adolescents, ages 9 to 18 over a one-year time period. The researchers compared the average of three 24-hour diet recalls to the average of two Youth/Adolescent Food Frequency Questionnaires. The authors define the validity of a food frequency questionnaire as being, “the degree to which the instrument measures the diet of the subjects it was designed to study". When nutrient intake was compared between the two methods, the Youth/Adolescent Food Frequency Questionnaire demonstrated to be an accurate measure in order to assess an adolescent's diet over the past year (77).

In 2001, a food checklist was validated as part of the Child and Adolescent Trial for Cardiovascular Health (CATCH). The food checklist is similar to a FFQ in that it was developed to provide a brief, inexpensive, and easily administered measure of the diet. More specifically, the food checklist was developed to target 3 nutrients: total fat, saturated fat, and sodium. It consists of 40 items including 30 foods, 2 beverages, and 8 condiments. The criterion measure for validation was a 24-hour diet recall. Following extensive analysis, the researchers conclude that the food checklist is a reliable and valid assessment tool for measuring fat, saturated fat, and sodium in adolescents (91).

Vereecken et al. (92) validated a Young Adolescents' Nutrition Assessment on computer. The Young Adolescents' Nutrition Assessment on computer is a computerized 24-hour diet recall designed for children and adolescents aged 11 years and older. The program consists of one 24-hour diet recall made up of six meal occasions, embedded questions, and a selection of over 400 food items. In order to validate the program, two studies were initiated with a total of 145 participants. Study one compared the program to a one-day food record and study two compared the program with a 24-hour diet recall interview. Results indicate that the Young Adolescents' Nutrition Assessment on computer had significantly higher values for nutrient and energy intake when compared to the food record, however this was not seen when compared to the interview. The authors suggest that the Young Adolescents'

Nutrition Assessment on computer may be a useful method for collecting dietary information from adolescents when interviews cannot be completed (92).

In 2007, an adolescent web-based food frequency questionnaire was tested for validity and reproducibility. The questionnaire has been developed to be cost effective and easy to administer. The questionnaire contains 15 food groups including beverages, snacks, and sauces, among others. The participants were a selected group of 12 to 18 year olds (n= 104). The FFQ data was compared to 3-day estimated food records. The data suggests that most questions on the 15 food groups had acceptable reproducibility however, validation was only shown for select food groups: water, fruit, bread, and fish/eggs/meat. This indicates that further development and investigation is needed in order to establish a valid FFQ for an adolescent population (93).

Cullen et al. (94) tested the reliability and validity of a Block Kids Questionnaire. The Block Kids Questionnaire is a self-report of consumption based on 72 food/beverage items for a period of 7 days. The questionnaire uses 6 response categories, ranging from none to everyday. Additional questions were used in order to clarify response, such as cereal type. Visual aids were used for serving sizes on plates and bowls. Children and adolescents (n=83) aged 10-17 years old completed two 24-hour diet recalls by telephone in order to compare dietary intake to the Block Kids Questionnaire. The results suggest, that in general, the Block Kids Questionnaire has validity for some nutrients and may be most beneficial for adolescents older than age 12 years (94).

Foster et al. (95) developed and evaluated three different portion size assessment tools for use with children. This may be very helpful since previous work finds that portion size estimation is an issue with adolescent diet assessment (79). The aim of the study was to explore a child's perception and conceptualization of food portion sizes using three tools: child-specific food photographs, food models and a novel interactive portion size assessment system. The study included 200 children, ages 4-16 years old. The children were provided with foods of known weights and each portion size tool was evaluated during a 24-hour diet recall. The authors found the food models to be the least accurate of the three. The interactive portion size assessment system and the food photographs were reported as having potential for use with children, however the validity needs to be tested in a less controlled environment, such as a "real life" situation with actual food intakes (95).

In 2010, Ambrosini et al. (96) compared a standardized FFQ and a 3-day food record in adolescents for validity. Since FFQ's generate information about the general diet over a long period of time and food records are known to generate in-depth information about the diet over 3-4 days, a diet pattern association between the two methods was tested. In order to compare the data, each item that was recorded in the food record was matched with an FFQ item. The scientists found that the nutrient profiles of the dietary patterns between the two methods were similar. Two patterns were found when both methods of dietary assessment were used on adolescents (average age of 14 years old): a "healthy" and a "western" dietary pattern. Since similar diet patterns were found when both methods were used in adolescents, the authors conclude that these two dietary patterns are relatively valid in adolescents (96).

A study by Kobayashi et al. (97) looked to validate a food frequency questionnaire (CFFQ) for estimating habitual intake in children and adolescents. The author notes that it is not feasible to conduct 24-hour recall for multiple days in order to assess children's habitual dietary intake. This may be due to difficultly in obtaining accurate dietary information because children are known to lack knowledge about foods and cooking methods (97). Additionally, it has been reported that when FFQs are administered to adults, dietary intake is overestimated. The FFQ (CFFQ) developed for assessing the diets of children is a 75-item questionnaire which includes the average portion size consumed by 3 to 11 year olds and also individual foods as well as mixed dishes. In order to validate the CFFQ, it was compared to weighed dietary records and also to an adult FFQ. The participants were among two groups, 3-11 years old (YC) and 12-16 years old (AD) for a total of 103 children. The questionnaires for the children in the YC groups were completed by their parent/guardian while the AD group self-administered. The researchers found that the CFFQ may be a helpful tool for assessing habitual dietary intake for the children in the YC group, however it was found to underestimate dietary intake in the AD group (97).

Diet assessment methods have even been developed in order to analyze the intake of specific food groups. A FFQ was developed by Ross et al. (98) that specifically targets whole-grain cereal intake. However, a food group-FFQ specific for adolescents has not been found in the literature. Currently, methods are still being developed and validated in order to accurately measure the diets of adolescents.

Future Dietary Assessment Methods

Technological advances have led to the development of diet assessment and evaluation tools on mobile devices (99). A group at Purdue University has developed an application for a smart phone, which provides a unique vehicle for collecting dietary information. The diet assessment application uses an image analysis approach that involves the subject taking a picture, using the mobile device, of food before and after consumption. The researchers believe that this approach will lessen the burden of recalling intake that is necessary for traditional diet assessment methods and is known to be a limitation. The next step is to test the device for accuracy and subject proficiency (99).

Previous results found that adolescents prefer technology-based dietary assessment methods to traditional methods. It is known that adolescents tend to have less structured eating habits such as eating away from home, snacking a lot and problems with remembering what they consumed and so their burden of reporting diet intake may be greater than adults. Additionally, reporting serving sizes of foods and drinks consumed has also shown to be problematic for adolescents. In 2010, evidence-based development of the mobile telephone food record was reported. Seventy-eight adolescents taking part in a technological based summer camp were recruited to use the mobile telephone food record for one or two meals. The system, a mobile telephone with a built in camera, is used to capture images of food before and after consumption and sent to a server for evaluation. Next, the information from image analysis and volume estimation can be linked to a nutrient database in order to estimate energy and nutrients consumed, this information can then be sent to researchers or dietitians to be further analyzed (100).

In regard to ease of use, the majority of participants agreed that the software was easy to use. Moreover, the majority of participants were able to save images that included all of the foods and beverages in both before and after images for the first and second meal. It was also reported that the adolescents’ proficiency for capturing the image of everything consumed improved after the first use. In conclusion, the researcher states that results from this study will lend a hand in improving future diet assessment methods in adolescents (100).

CHAPTER 2
PURPOSE

Consuming adequate amounts of whole grain foods (at least 3 ounce servings per day) is important because the individual may acquire healthful benefits such as reducing the risk of developing chronic diseases and maintaining a healthy weight (2). Adolescents are a population known to under-consume (<1 serving per day) whole grains (62). Thus, the current study will quantify the amount of grains consumed by adolescents while receiving weekly groceries of whole- or refined-grain foods. For the research proposed here, targeted multi-pass 24-hour diet recalls were administered to assess the effects of providing grain foods to middle-school children. It is hypothesized that providing whole grains to adolescents will increase their daily consumption to the recommended amount.

CHAPTER 3
METHODS

Study Design

A six-week, randomized, controlled, parallel-arm study was carried out with 83 healthy adolescents, ages 11-16 years, enrolled in Westwood Middle School, Gainesville, FL (Figure 3-1). Participants were started on the protocol in three waves. Wave 1 was randomized February 8 through 11 (whole grain, n=21; refined grain, n=24). Wave 2 was randomized February 16 through 19 (whole grain, n=16; refined grain, n=16), and wave 3 was randomized on February 23 (whole grain, n=4; refined grain, n=2). Only one child from each household was enrolled to avoid having two children from the same household being randomized to two different treatment groups. If two students in the same household were interested in participating, the younger child was selected to participate. Institutional Review Board approval was obtained from the University of Florida IRB-01. Approval was also obtained by the Alachua County Public Schools and Westwood Middle School. Informed consent from one parent and assent from all study participants was obtained.

Inclusion / Exclusion Criteria after Obtaining Consent and Assent

Participants were included if they were willing to eat three different study foods each day for six weeks. Subjects were excluded if they i) were taking any medications for constipation or diarrhea, receiving antibiotics during the four weeks prior to randomization, or consuming probiotics or more than three servings per week of yogurt; ii) had any diseases or illnesses such as gastrointestinal disease (gastric ulcers, Crohn’s, ulcerative colitis, etc.), diabetes, kidney disease or immune-modulating diseases (HIV, AIDS, autoimmune, hepatitis, cancer etc.), or any food allergies.

Pre-Baseline and Baseline

All participants were given a disposable soup-sized bowl marked in quarter cup increments. Participants were told to use these bowls to estimate the serving size of cereal, pasta, rice and any other study foods provided. These same bowls were used during the targeted 24-hour diet recalls to more accurately describe serving sizes. The participant or family were contacted via phone or email to remind them of study appointments, schedule new appointments, discuss study procedures, deliver study foods, and obtain the 24-hour diet recalls. Pictures were taken of each student to keep in the subject file for identification purposes. In the week before randomization height, weight, age, and body mass index was obtained to determine BMI percentile for age.

Trained study coordinators administered two, targeted multiple-pass 24-hour diet recalls. Participants were asked to list all of the foods and beverages they consumed in the previous 24 hours (midnight to midnight); but specific information (i.e., serving size, whole grain vs. refined grain, method of preparation, etc.) was only obtained for grain products, fruits, and vegetables. Time was limited for acquiring diet information; therefore to obtain the most accurate measure, only extensive information on grains, fruits, and vegetables consumed was obtained. Cups, disposable soup bowls, and food diagrams were used to help determine serving size. A measurement guide and procedure booklet was created for the interviewers to use while administrating the diet recall. The booklet included a step-by-step procedure of the multi-pass method, pictures of foods and dimensions, measurement techniques, and pictures of grain foods that were provided to the children.

Investigators worked closely with the food service director at the middle school to obtain menus, recipes, and lists of whole grain foods provided during the study period.

On the first day of the study, participants were randomized and received their study food for the week.

Randomization

Participants were proportionally stratified based on BMI (<95th BMI percentile for age or BMI <30, $\geq$95th BMI percentile for age or BMI $\geq$30) and randomized into two groups (i.e., whole grain or refined grain). This study was not blinded but study groups were denoted as green (whole grain) or white (refined grain) to de-emphasize the treatments. Participants were randomized via sealed envelopes to receive whole grain foods or refined grain foods. The stratification and randomization schemes were generated by the study statistician, who did not have direct contact with any subjects.

Treatment

Foods, such as pasta, rice, bread, cereal, and pancake mix were provided for the study subject's family for the six-week study period. Four rotating weekly food packages were planned (Table 3-1, Table 3-2, Table 3-3, and Table 3-4). Foods for each treatment group's weekly package were matched as close as possible based on food type and brand between the groups. For example, in whole grain package 1, whole grain pasta was included and in refined grain package 1, regular pasta was included. Parents were encouraged to pick up the weekly food packages before or after school; however, approximately half of all weekly food packages were delivered to the subjects' homes. Study staff were available at the school (e.g., before and after school or during home room or lunch) to distribute study snack foods and assist study participants with questionnaire completion. Two single-servings of whole grain cereal packs (whole grain group) or 100-kcal cookie packs (refined-grain group) were distributed to subjects on school days. Students were asked to consume at least three different study foods each day. There was no maximum limit for daily study food consumption. Study staff emphasized replacing normally consumed grain products with study foods rather than adding more food, to ensure energy balance was maintained. The average nutrient information per ounce equivalent for the whole grain and refined grain packages is listed in Table 3-5.

Ounce Equivalents

USDA ChooseMyPlate guidelines for ounce equivalents were used in order to quantify the ounces of grains per serving consumed by the participants. According to ChooseMyPlate, one slice of bread, one cup of ready-to-eat cereal, or ½ cup of cooked rice, cooked pasta, or cooked cereal is considered one-ounce equivalent from the grains group (Table 3-5). If a grain food consumed by a participant was not included in the ChooseMyPlate guidelines for ounce equivalents, the grain content was determined by entering the food into myfoodapedia.gov (61). Lastly, if the food was not found in myfoodapedia.gov, the grain content was determined using calculations based on the food label. Some examples of foods that were not found on myfoodapedia.gov are cookies, cakes, brownies, and granola bars. For these, total carbohydrate as well as sugar from carbohydrate was calculated to find the grain content. Since all grain ounce equivalents are based from one slice of bread and the average slice of bread contains 20% of its carbohydrate from sugar and contains 15 g of carbohydrates, the grain content was determined by multiplying the grams of carbohydrate by the amount of sugar found in one slice of bread (20%). Next, that amount was subtracted from the sugar listed on the food label. Foods with greater than 20% carbohydrate from sugar will be subtracted from total carbohydrate and divided by 15 g.

For example: Granola Bar

23 g Carbohydrate (CHO)   12 g sugar
23 g CHO X 0.2= 4.6 g
12 g sugar – 4.6 g = 7.4 g = sugar content of granola bar based on one slice of bread
23 g CHO- 7.4 g = 15.6 g CHO
15.6 g/ 15 = 1.04 ounce equivalents

Statistical Analyses

Data were analyzed for intent to treat subjects (included all subjects enrolled in the study). Daily dietary intake of grains during the pre-baseline period was averaged for each subject to provide the average “pre-baseline” intake. Similarly, daily intake from study and non-study grain products during the six-week intervention period was averaged for each subject to provide a single “treatment” intake. The effect of gender, race/ethnicity, study period (pre-baseline vs. treatment), treatment group, and the interactions between treatment group and the other main effects on average daily intake of whole-, refined-, total-grain, fruit and vegetable intake was examined. Data were analyzed using mixed effects linear models with the above-mentioned variables as fixed effects and student as a random effect to account for the repeated measurements on each student (pre-baseline vs. treatment). Residuals were checked to ensure the assumptions of normality and constant variance were met. When fixed effect variables or their interactions were significant (α =0.05), treatment means were tested using the Tukey-Kramer adjustment to control the family-wise error rate at α=0.05.

Daily and Weekly Measures

Participants were given study workbooks with tear-out pages for daily logs (Appendix A) and asked to come to the designated study area (i.e., school trophy hall) each school day to drop off their completed logs. The daily logs asked about the consumption of study foods. Each week the subject met with a study coordinator to be weighed and to provide a targeted 24-hour diet recall.

Final Study Week

During the last week of the study intervention, one 24-hour targeted diet recall was obtained from each participant. On the last day of the study, height and weight was also reassessed.

Incentives

In addition to study foods, subjects received coupons with a total of seven coupons possible: one coupon for completing the pre-study week and six additional coupons for completing each of the six study weeks. Each week they participated they received another coupon. At the end of the study, the subjects traded the coupons for one prize. There were seven levels of prizes of increasing dollar value to approximately $100 for seven coupons (i.e., for completing the study).

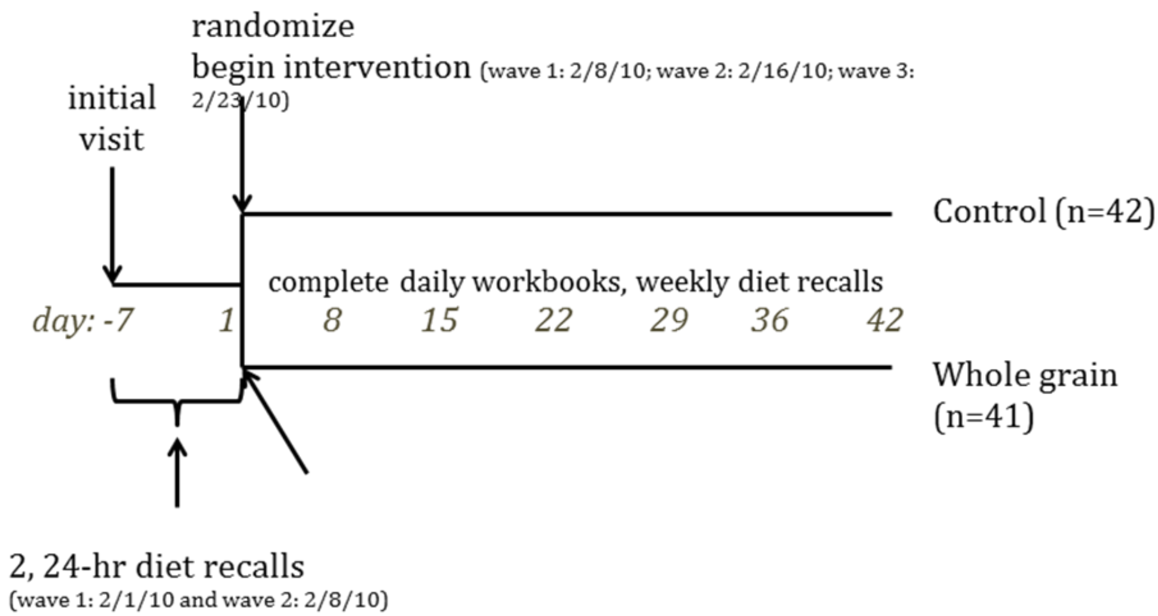

Figure 3-1. Study design

Table 3-1. Foods provided during weeks 1 and 5 for subjects in wave 1, week 4 for wave 2 and week 3 for wave 3.

Whole Grain Package #1	Fiber/Ounce equivalent	Whole Grain/Ounce equivalent	Refined Package #1	Fiber/Ounce equivalent
Honey Nut Cheerios (General Mills)	3 g	18.7 g	Frosted Flakes (Kellogg's)	0.7 g
100% Whole Wheat Bread (Arnold) (2 loaves/week)	3 g	22 g	Country White Bread (Arnold) (2 loaves/week)	1 g
Whole Wheat Rotini (Barilla)	2 g	14 g	Rotini Pasta (Barilla)	1 g
Natural Brown Rice (Publix)	1 g	28 g	Long Grain Enriched Rice (Publix)	0.5 g
Ready Brown Rice (Uncle Ben's)	1 g	25.5 g	Ready White Rice (Uncle Ben's)	0.5 g
Blueberry Cereal Bars (Nutri-Grain) (Kellogg's)	1 g	8 g	Fruit and Grain Blueberry Bars (Publix)	2 g
Whole Wheat Blend Pancake Mix (Aunt Jemima)	1 g	6.5 g	Original Complete Pancake Mix (Aunt Jemima)	0 g
Oats and Honey Granola Bars (Nature Valley) (General Mills)	1 g	14 g	Rice Krispies Treats Variety Pack (Kellogg's)	0 g
Sun Chips Harvest Cheddar (Frito Lay)	2 g	14.4 g	Mini Pretzels (Sun Snyder of Hanover)	1 g
Whole Grain Goldfish (Pepperidge Farm)	1 g	11.2 g	Goldfish (Pepperidge Farm)	1 g
Nesquik Cereal Packs (General Mills)	1 g	9.7 g	100 Calorie Right Bites (Kellogg's)	1 g
			Macaroni and Cheese (Kraft)	1 g

Table 3-2. Foods provided during weeks 2 and 6 for subjects in wave 1, weeks 1 and 5 for wave 2 and week 4 for wave 3.

Whole Grain Package #2	Fiber/Ounce equivalent	Whole Grain/Ounce equivalent	Refined Grain Package #2	Fiber/Ounce equivalent
Reese's Puffs Cereal (General Mills)	2 g	14.5 g	Special K (Kellogg's)	2.6 g
Select Wheat Sandwich Rolls (Arnold)	3 g	10 g	Sandwich Rolls with Sesame Seeds (Arnold)	1 g
100% Whole Wheat Bread (Arnold)	3 g	22 g	Country White Bread (Arnold)	1 g
Whole Wheat Rotini (Barilla)	2 g	14 g	Rotini Pasta (Barilla)	1 g
Whole Grain Macaroni and Cheese (Kraft)	1 g	14.5 g	Macaroni and Cheese (Kraft)	1 g
Ready Brown Rice (Uncle Ben's)	1 g	25.5 g	Ready White Rice (Uncle Ben's)	0.5 g
Strawberry Cereal Bars (Nutri-Grain)(Kellogg's)	1 g	8 g	Fruit and Grain Strawberry Bars (Publix)	2 g
Instant Oatmeal Packets: Flavor Variety (Quaker)	2 g	25 g	Instant Cream of Wheat Variety Pack (B & G Foods)	1 g
Nature Valley Granola Bars Variety Pack (General Mills)	1 g	14 g	Rice Krispies Treats Variety Pack (Kellogg's)	0 g
Tostitos Restaurant Style Tortilla Chips (Frito Lay)	1 g	6 g	Mini Pretzels (Sun Snyder of Hanover)	1 g
Whole Grain Goldfish (Pepperidge Farm)	1 g	11 g	Goldfish (Pepperidge Farm)	1 g
Orville Redenbacher Popcorn	3.5 g	35 g	Rice Cake Snacks Ranch Flavor (Quaker)	0 g
Nesquik Cereal Packs (General Mills)	1 g	9.7 g	100-Calorie Right Bites	1 g

Table 3-3. Foods provided during week 3 for subjects in wave 1, weeks 2 and 6 for wave 2 and weeks 1 and 5 for wave 3.

Whole Grain Package #3	Fiber/Ounce equivalent	Whole Grain/Ounce equivalent	Refined Grain Package #3	Fiber/Ounce equivalent
Total Cinnamon Crunch (General Mills)	4 g	8 g	Rice Krispies (Kellogg's)	0 g
Nature's Own Honey Wheat Bagels (Flower Foods)	1 g	4 g	Nature's Own Original Bagels (Flower Foods)	0.6 g
100% Whole Wheat Bread (Arnold)	3 g	22 g	Country White Bread (Arnold)	1 g
Whole Grain Macaroni and Cheese (Kraft)	1 g	14.5 g	Macaroni and Cheese (Kraft)	1 g
Ready Brown Rice (Uncle Ben's)	1 g	25.5 g	Ready White Rice (Uncle Ben's)	0.5 g
Nutri-Grain Apple Cinnamon Cereal Bars (Kellogg's)	1 g	8 g	Fruit and Grain Apple Cinnamon Bar (Publix)	2 g
Instant Oatmeal Packets: Flavor Variety (Quaker)	2 g	25 g	Instant Cream of Wheat Variety Pack (B & G Foods)	1 g
Nature Valley Granola Bars Variety Pack (General Mills)	1 g	14 g	Rice Krispies Treats Variety Pack (Kellogg's)	0 g
Original Sun Chips (Frito Lay)	2 g	14 g	Mini Pretzels (Sun Snyder of Hanover)	1 g
Whole Wheat Ritz Crackers (Nabisco World)	1 g	5 g	Reduced Fat Ritz Crackers (Nabisco World)	0 g
Nesquik Cereal Packs (General Mills)	1 g	9.7 g	100-Calorie Right Bites (Kellogg's)	1 g

Table 3-4. Foods provided during week 4 for subjects in wave 1, week 3 for wave 2 and weeks 2 and 6 for wave 3.

Whole Grain Package #4	Fiber/Ounce equivalent	Whole Grain/Ounce Equivalent	Refined Grain Package #4	Fiber/Ounce equivalent
Frosted Mini Wheat's (Maple and Brown Sugar) (Kellogg's)	5 g	46 g	Cocoa Krispies (Kellogg's)	1 g
Whole Wheat Tortillas (Mission)	1 g	15 g	Flour Tortillas (Mission)	1 g
100% Whole Wheat Bread (Arnold)	3 g	22 g	Country White Bread (Arnold)	1 g
Whole Grain Thin Spaghetti (Barilla)	3 g	14 g	Thin Spaghetti (Barilla)	1 g
Ready Brown Rice (Uncle Ben's)	1 g	25.5 g	Ready White Rice (Uncle Ben's)	0.5 g
Nutri-Grain Mixed Berry Cereal Bars (Kellogg's)	1 g	8 g	Fruit and Grain Mixed Berry Bars (Publix)	2 g
Instant Oatmeal Packets: Flavor Variety (Quaker)	2 g	25 g	Instant Cream of Wheat Variety Pack (B & G Foods)	1 g
Nature Valley Granola Bars Variety Pack (General Mills)	1 g	14 g	Rice Krispies Treats Variety Pack (Kellogg's)	0 g
Tostitos Restaurant Style Tortilla Chips (Frito Lay)	1 g	8 g	Mini Pretzels (Sun Snyder of Hanover)	1 g
Whole Grain Goldfish (Pepperidge Farm)	1 g	11 g	Goldfish (Pepperidge Farm)	1 g
Nesquik Cereal Packs (General Mills)	1 g	9.7 g	100-Calorie Right Bites (Kellogg's)	1 g

Table 3-5. Specific amounts that count as 1-ounce equivalent of grains towards daily-recommended intake.

Grain Food	Type of Grain	Amount that counts as 1 ounce equivalent of grains	Common portions and ounce equivalents
Bagels	WG*: whole wheat RG*: plain, egg	1 "mini" bagel	1 large bagel = 4 ounce equivalents
Biscuits	(baking powder/ buttermilk—RG*)	1 small (2" diameter)	1 large (3" diameter) = 2 ounce equiv
Bulgur	cracked wheat (WG*)	½ cup cooked	
Cornbread	(RG*)	1 small piece (2 ½" x 1 ¼" x 1	1 medium piece (2 ½" x 2 ½" x 1 ¼") = 2 ounce equivalents
Crackers	WG*: 100% whole wheat, rye	5 whole wheat crackers 2 rye crispbreads	
	RG*: saltines, snack crackers	7 square or round crackers= 1 ounce equivalent	
English muffins	WG*: whole wheat RG*: plain, raisin	½ muffin 1 muffin = 2 ounce equivalents	1 muffin = 2 ounce equivalents
Muffins	WG*: whole wheat RG*: bran, corn, plain	1 small (2 ½" diameter)	1 large (3 ½" diameter) = 3 ounce equivalents
Oatmeal	(WG)	½ cup cooked 1 packet instant 1 ounce dry (regular or quick)	
Pancakes	WG*: Whole wheat, buckwheat RG*: buttermilk, plain	1 pancake (4 ½" diameter) 2 small pancakes (3" diameter)	3 pancakes (4 ½" diameter) = 3 ounce equivalents
Popcorn	(WG*)	3 cups, popped	1 microwave bag, popped = 4 ounce equivalents
Ready-to-eat breakfast cereal	WG*: whole wheat RG*: enriched, durum	1 cup flakes or rounds 1 ¼ cup puffed	1 cup cooked = 2 ounce equivalents

Table 3-5. Continued.

Grain Food	Type of Grain	Amount that counts as 1 ounce equivalent of grains	Common portions and ounce equivalents
Rice	WG*: brown, wild RG*: enriched, white, polished	½ cup cooked 1 ounce dry	1 cup cooked = 2 ounce equivalents
Pasta--spaghetti, macaroni, noodles	WG*: whole wheat RG*: enriched, durum	½ cup cooked 1 ounce dry	1 cup cooked = 2 ounce equivalents
	WG*: whole wheat, whole grain corn RG*: Flour, corn	1 small flour tortilla (6" diameter) 1 corn tortilla (6" diameter)	1 large tortilla (12" diameter) = 4 ounce equivalents

CHAPTER 4
RESULTS

Subject Demographics and Characteristics

From a population of 970 students, 196 were screened and 91 were consented (Figure 4-1). Of these potential subjects, eight were excluded. Four of the subjects did not meet inclusion/exclusion criteria (three changed schools, one started on antibiotics) and four were no longer interested in participating. Forty-two subjects were randomized to the refined-grain group and 41 to the whole-grain group. All subjects completed the 8 week study period. There were no differences between groups in regards to gender, age, grade in school, race/ethnicity, the percentage of subjects classified as under or healthy weight (<85^{th} percentile BMI for age), overweight (85^{th} to <95^{th} percentile), or obese ($\geq 95^{th}$ percentile), participation in school breakfast or lunch, preference for study foods, the percentage of subjects who walk or bike to school or participate in organized sports/activities or number of days of targeted 24-hour diet recalls of intent to treat (ITT) subjects. However, fewer subjects in the whole grain group took a physical education class in school (Table 4-1). The average number of 24-hour targeted diet recalls obtained during the pre-baseline and treatment periods were not significantly different between the treatment groups (Table 4-2). The percentage of school days (i.e., Monday through Friday) assessed by the targeted 24-hour recalls was compared for all subjects across gender, treatment group, study period (pre-baseline vs. treatment), and race/ethnicity. The percentage of the school day intakes did not depend on gender, race/ethnicity, the pre-baseline or treatment periods or treatment group for the ITT analysis (Table 4-1).

Nutrient information of the food packages was obtained using Food Processor software based on ounce equivalents. Foods in the whole grain packages had significantly higher total fat, dietary fiber, sugar, and protein and significantly lower sodium than foods in the refined grain packages. On average, the whole grain packages provided 16 grams of whole grain per ounce equivalent (Table 4-3). Figure 4-2 and 4-3 represent the most commonly consumed food item provided in the food packages.

Total Grain Intake

Dietary intakes of grains during the pre-baseline period were averaged for each subject to provide the average "pre-baseline" intake. Intakes during the six-week intervention period were averaged for each subject to provide a single "treatment" intake. The effect of gender, race/ethnicity, study period (pre-baseline vs. treatment), and treatment group on average daily intake of all grains (ounce equivalents) was examined for all subjects. The average daily ounces of grain represents intake of refined and whole grains from all foods (i.e., study and non-study foods). Gender, treatment group, study period, race/ethnicity and the interaction between treatment group and race/ethnicity had a significant effect on the average daily intake of total grain ounce equivalents (Table 4-4). Males consumed significantly more ounce equivalents of total grains than females (P=0.0026) across both groups and treatment periods (pre-baseline/treatment period) (Figure 4-4). However, there was not a significant interaction between gender and treatment group or gender, treatment group, and study period (i.e., pre-baseline vs. treatment periods). This suggests that males and females did not eat different amounts of total grains when they were randomized to receive refined-grain versus whole-grain foods.

Total grain intake was significantly lower across study periods in subjects eventually randomized to the whole-grain group (P=0.0063) (Figure 4-5), and all subjects consumed more grains during the study period (P=0.0014) regardless of treatment group (Figure 4-6). There was no treatment group by study period interaction (P=0.6878) suggesting that total grain intake was not different between subjects in the refined-grain and whole-grain groups during the pre-baseline or treatment periods (data not shown).

Total grain intake was significantly different across race/ethnicity (Figure 4-7). Tests of effect slices for group*race/ethnicity sliced by race/ethnicity or by group (Fisher's Least Significant Difference Tests) showed that Hispanic and "Other" subjects took in fewer grains when they were randomized to the whole-grain group. The "other" category consisted of Asians (n=2), mixed White/African American (n=4) and subject-defined as other (n=2).

Refined-Grain Intake

Dietary intakes of grains during the pre-baseline period were averaged for each subject to provide the average "pre-baseline" intake. Intakes during the six-week intervention period were averaged for each subject to provide a single "treatment" intake. The effect of gender, race/ethnicity, study period (pre-baseline vs. treatment periods), and group on average daily intake of refined grain (ounce equivalents) was examined for all subjects. The average daily ounce equivalents of refined grain represents intake from all foods (i.e., study and non-study foods). Gender, treatment group and the interaction between treatment group and study period significantly influenced the average daily intake of refined grains (Table 4-7). Males took in significantly more refined grains than females across treatment groups and periods

(Figure 4-8). Pair-wise comparisons show that participants randomized to the refined grain group had a significantly higher average daily intake of refined grains compared to those randomized to the whole-grain group (data not shown).

During the pre-baseline period, subjects consumed on average 4.1±0.2 oz. (LS means±SE) equivalents of refined grains, and there was no difference in intakes by subjects eventually randomized to the whole-grain versus refined-grain groups. Subjects in the refined-grain and whole-grain groups significantly increased and decreased their average daily intake of refined grains, respectively during the treatment period compared with the pre-baseline period (Figure 4-9). There were no differences in the average daily intake of refined grains among ethnic and racial groups.

Whole Grain Intake

Dietary intakes of grains during the pre-baseline period were averaged for each subject to provide the average "pre-baseline" intake. Intakes during the six-week intervention period were averaged for each subject to provide a single "treatment" intake. The effect of gender, race/ethnicity, study period (pre-baseline vs. treatment periods), and treatment group on average daily intake of whole grain (ounce equivalents) was examined for all subjects. The average daily ounce equivalents of whole grain represents intake from all foods (i.e., study and non-study foods). Treatment group, study period, race/ethnicity and the interaction between group and study period had a significant effect on the average daily intake of ounce equivalents of whole grain (Table 4-8).

Subjects were consuming on average 1.0±0.2 oz. equivalents (LS mean±SE) of whole grain during the pre-baseline period. There was no difference in average intake of whole grain (ounce equivalents) during the pre-baseline period by subjects eventually randomized to the refined-grain or whole-grain groups. Average intake of whole grain was significantly higher in those participants randomized to the whole-grain treatment group compared to the refined-grain treatment group. Subjects randomized to the whole-grain or refined-grain group significantly increased and decreased, respectively, their average intake of whole grains. Average intake of whole grains was significantly greater during the treatment period across all participants. Also, the treatment group and treatment period interaction was significantly affected by whole-grain intake, resulting in those participants randomized to the whole-grain group consuming on average 3.6 ounce-equivalents of whole grains per day (Figure 4-10).

White participants consumed significantly higher amounts of whole grains when compared to Black/African American, Hispanic/Latino, and other participants. Additionally, whole grain intake by treatment group across the pre-baseline and treatment periods of different races and ethnicities was significantly different (Figure 4-11). The table inset shows the tests of effect slices for group*race/ethnicity sliced by race/ethnicity or group using the Fisher's Least Significant Difference Test. These analyses suggest that differences in whole-grain intake (ounce equivalents) by African American, White, and "Other" subjects is due to difference in intake of these subjects in the whole-grain group. The "other" category consisted of Asians (n=2), mixed White/African American (n=4) and subject-defined as other (n=2).

Fruit Intake

Dietary intakes of fruit (cup equivalents as indicated in ChooseMyPlate) during the pre-baseline period were averaged for each subject to provide the average "pre-baseline" intake. Intakes during the six-week intervention period were averaged for each subject to provide a single "treatment" intake. The following were counted as fruit intake:

fruits and 100% fruit juice on a cup-for-cup basis, and fruit punch or lemonade 2 cups equal a ¼ cup equivalent). The effect of treatment group, gender, race/ethnicity, and study period (pre-baseline vs. treatment) and the interactions among these effects on fruit intake (cup equivalents) was tested for all subjects (Table 4-11). Only the gender by group interaction was significant (Figure 4-12). Fruit intake during the pre-baseline and treatment period for both treatment groups was less than 1 cup/day and was not changed with either treatment.

Vegetable Intake

Dietary intakes of vegetables (cup equivalents as indicated in ChooseMyPlate) during the pre-baseline period were averaged for each subject to provide the average “pre-baseline” intake. Intakes during the six-week intervention period were averaged for each subject to provide a single “treatment” intake. The following were counted as vegetable intake: whole vegetables, vegetable juice, salsa, ketchup, pizza sauce, pickles, French fries, and potatoes. The effect of treatment group, gender, race/ethnicity, and study period (pre-baseline vs. treatment) and the interactions among these effects on vegetable intake (cup equivalents) was tested for all subjects (Table 4-12). Only the gender was significant (Figure 4-13). Vegetable intake during the pre-baseline and treatment period for both treatment groups was less than 1 cup/day and was not changed with either treatment.

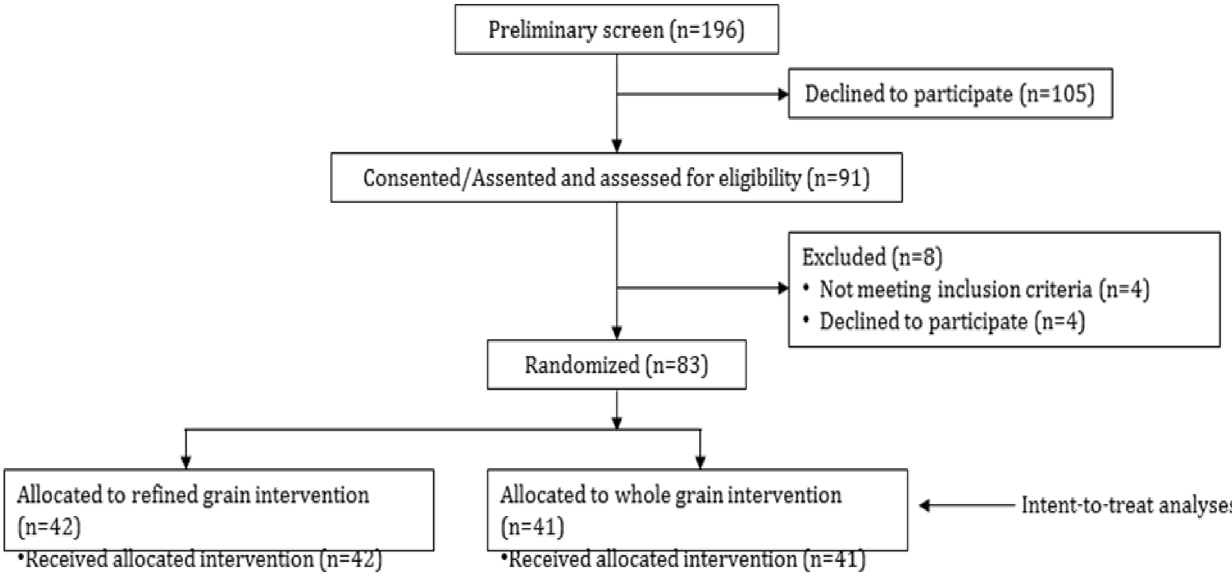

Figure 4-1. Flow chart of subject recruitment, allocation and analyses.

Table 4-1. Subject characteristics and dietary intake data.

	Refined Grain Group (n=42)	Whole Grain Group (n=41)	*P* value
Gender (M/F)	25/17	23/18	0.826
Age in years (median [25^{th}, 75^{th}])	12.9 (11.8, 13.5)	12.3 (11.7, 13.5)	0.503
Grade in school			0.199
6^{th} (n[%])	19 (45%)	22 (54%)	
7^{th} (n[%])	15 (36%)	9 (22%)	
8^{th} (n[%])	8 (19%)	10 (24%)	
Race/ethnicity (n[%])			0.213
White	18 (43%)	20 (49%)	
Black/African American	14 (33%)	12 (29%)	
Hispanic/Latino	6 (14%)	5 (12%)	
Mixed/Other[1]	4 (10%)	4 (10%)	

Table 4-1. Continued.

	Refined Grain Group (n=42)	Whole Grain Group (n=41)	*P* value
Baseline BMI percentiles for age (n[%])			0.492
< 85th (under or healthy weight)	23 (55%)	23 (56%)	
85th – 94th (overweight)	10 (24%)	6 (15%)	
≥95th (obese)	9 (21%)	12 (29%)	
Activity (n[%])			
Walks or bikes to school	5 (12%)	5 (12%)	1.000
Daily physical education class	19 (45%)	5 (12%)	0.001
Participates in organized sports/activities	14 (33%)	21 (51%)	0.122
School meals (d/wk, mean±SEM)			
Participates in school breakfast	0.7±0.2	0.5±0.2	0.690
Participates in school lunch	4.0±0.3	3.4±0.3	0.216
Preferences for foods in weekly food packs			
Foods would eat (out of 9, median [25th, 75th])	8 (7, 9)	8 (8, 9)	0.268
Targeted 24-hour diet recalls			
Number of days (median [25th, 75th])			
Pre-baseline	3 (2, 3)	3 (2, 3)	0.181
Treatment	5 (5, 5)	5 (5, 6)	0.362
Dietary intake recorded for school days			0.6552
Pre-baseline (%, LSmean±SEM)	73±5	75±5	
Treatment (%, LSmean±SEM)	65±5	72±5	
Total grain intake (oz eq/day, LSmean±SEM)			0.6883
Pre-baseline	5.7±0.4	4.4±0.4	
Treatment	6.7±0.4	5.7±0.4	

Table 4-1. Continued

	Refined Grain Group (n=42)	Whole Grain Group (n=41)	*P* value
Whole grain intake (oz eq/day, LSmean±SEM)			<0.00014
Pre-baseline	1.3±0.2	0.6±0.2	0.098
Treatment	0.3±0.25	3.5±0.25	<0.0001
Refined grain intake (oz eq/day, LSmean±SEM)			<0.00016
Pre-baseline	4.4±0.3	3.9±0.4	0.681
Treatment	6.5±0.35	2.1±0.35	<0.0001

Table 4-2. The average number of targeted 24-hour recalls obtained during the pre-baseline and treatment periods for the intent-to-treat subjects

	Refined-Grain Group (n=42)	Whole-Grain Group (n=40 for baseline, 41 for intervention)	P value
Number of recalls/subject (mean+SEM)			
Pre-baseline period	2.6±0.1	2.4±0.1	0.166
Treatment period	5.2±0.1	5.2±0.1	0.565

Table 4-3. Average caloric, nutrient, and fiber content per ounce equivalent of whole-grain and refined grain foods provided in the weekly food packages.

Nutrient	Refined Grain	Whole Grain	*P* value
Weight (g)	34.8±0.6	35.6±0.4	0.329
Whole grain (g)	0	16±1	0.029
Calories	114±2	119±3	0.260
Total fat (g)	1.7±0.1	2.5±0.2	0.019
Saturated fat (g)	0.4±0.03	0.4±0.1	0.343
Sodium (mg)	158±3	122±12	0.029
Carbohydrate (g)	22.1±0.5	21.1±0.4	0.162
Dietary fiber (g)	0.9±0.04	1.8±0.04	<0.001
Sugar (g)	4.3±0.3	5.3±0.2	0.034
Protein (g)	2.6±0.1	3.0±0.04	0.007
Vitamin A (IU)	262±42	169±18	0.088
Vitamin C (mg)	2.0±0.3	3.2±1.3	0.441
Calcium (mg)	41±2	67±25	0.355
Iron (mg)	2.3±0.2	2.2±0.3	0.829

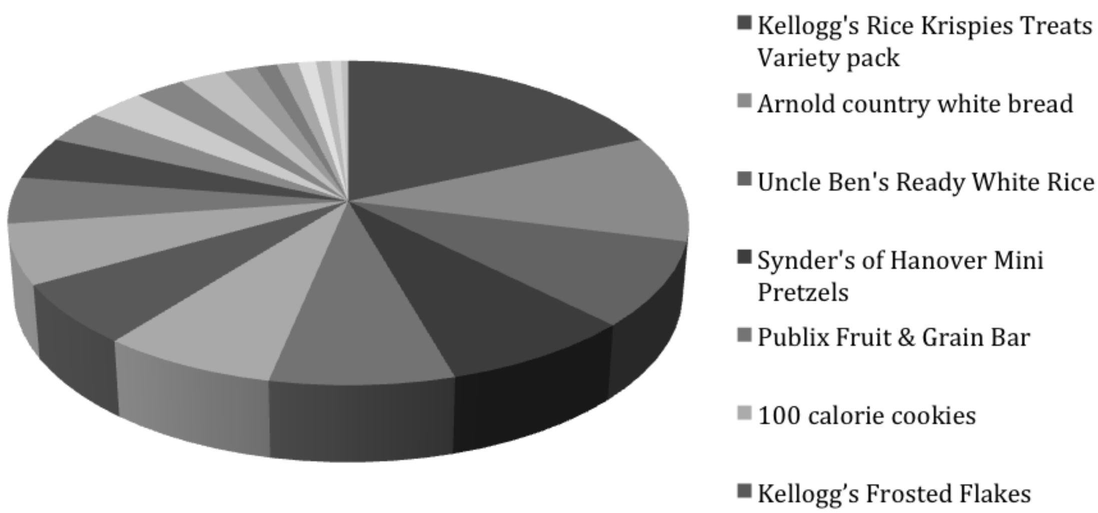

Figure 4-2. Ranking of most common refined-grain package foods consumed.

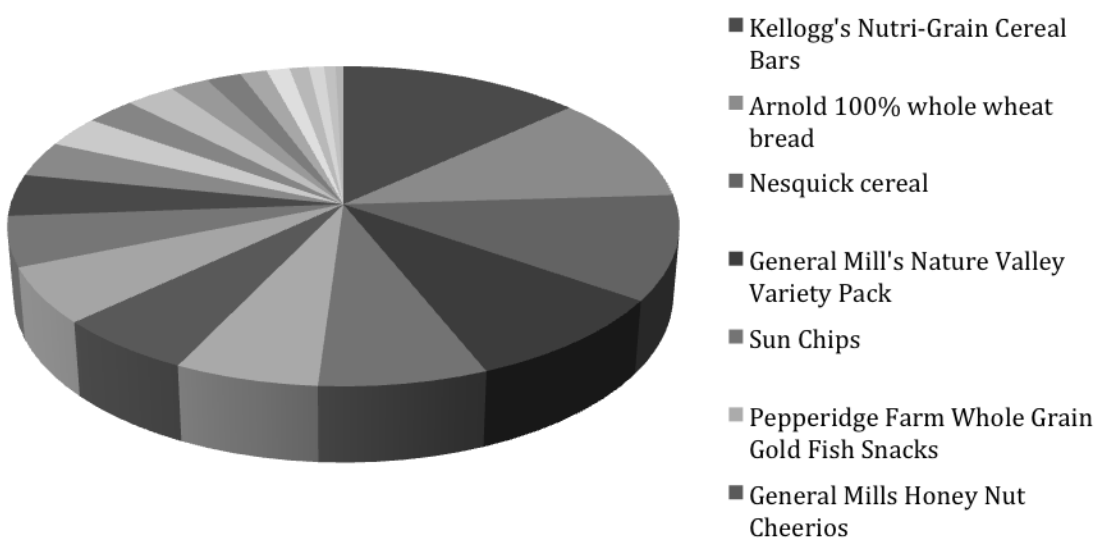

Figure 4-3. Ranking of most common whole-grain package foods consumed.

Table 4-4. The main effects of gender, group, study period, race/ethnicity, and their interactions on average daily intake of all grains.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Gender	1	151	9.36	0.0026
Group (RG, WG)	1	151	7.68	0.0063
Gender*Group	1	151	0.22	0.6417
When (pre-baseline, treatment)	1	151	10.54	0.0014
Gender*when	1	151	1.75	0.1880
Group*when	1	151	0.16	0.6878
Gender*Group*when	1	151	0.62	0.4307
Race/ethnicity	3	151	3.43	0.0186
Group*Race/ethnicity	3	151	2.87	0.0383

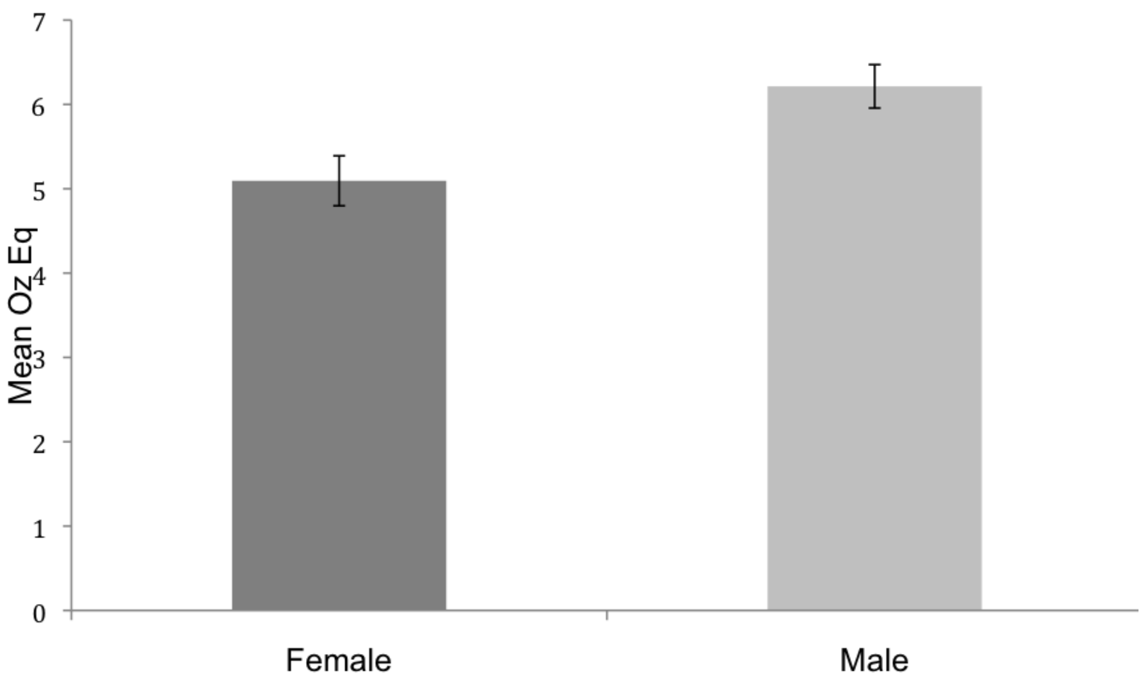

Figure 4-4. The least squares means for the average daily intake of all grains by females and males across treatment groups and the pre-baseline and treatment periods. The effect of gender on total grain intake was significant (P=0.0026).

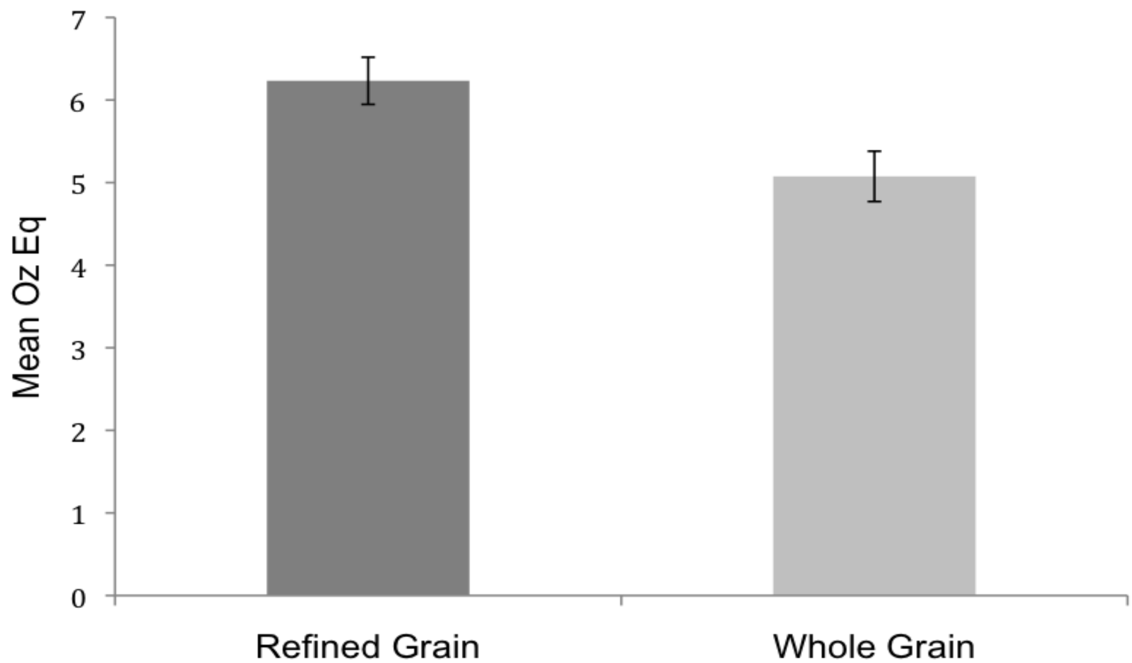

Figure 4-5. The least squares means for the average daily intake of all grains by subjects in the refined-grain or whole-grain groups across the pre-baseline and treatment periods. The effect of treatment group on total grain intake was significant (P=0.0063).

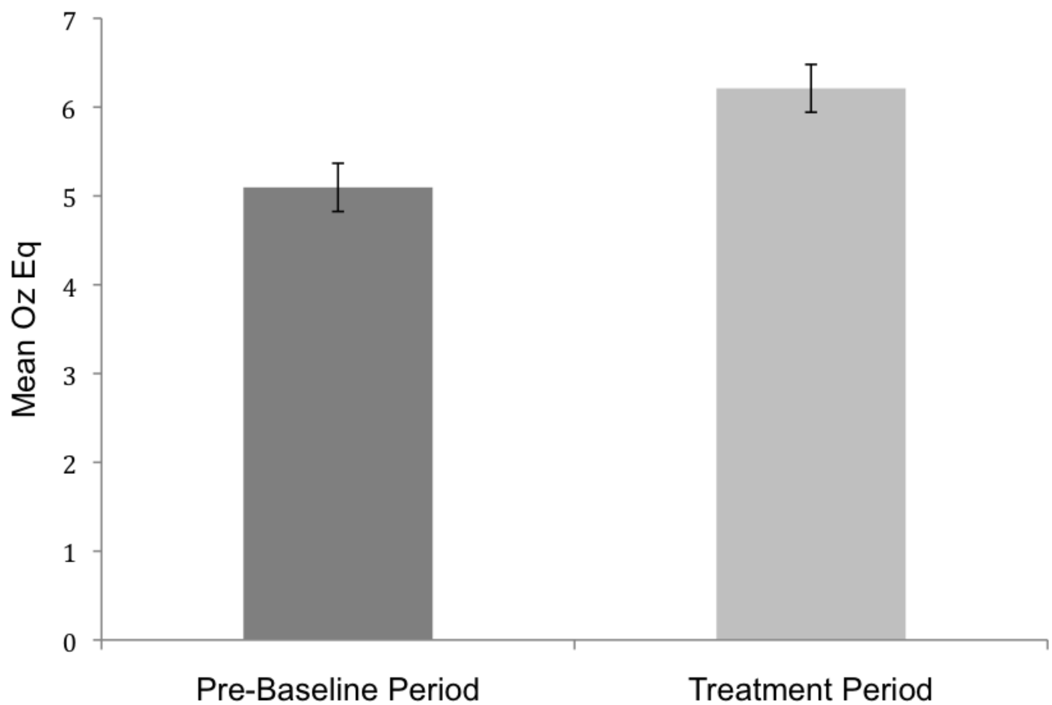

Figure 4-6. The least squares means for the average daily intake of all grains by all subjects during the pre-baseline and treatment periods across treatment groups. The effect of study period on total grain intake was significant (P=0.0014).

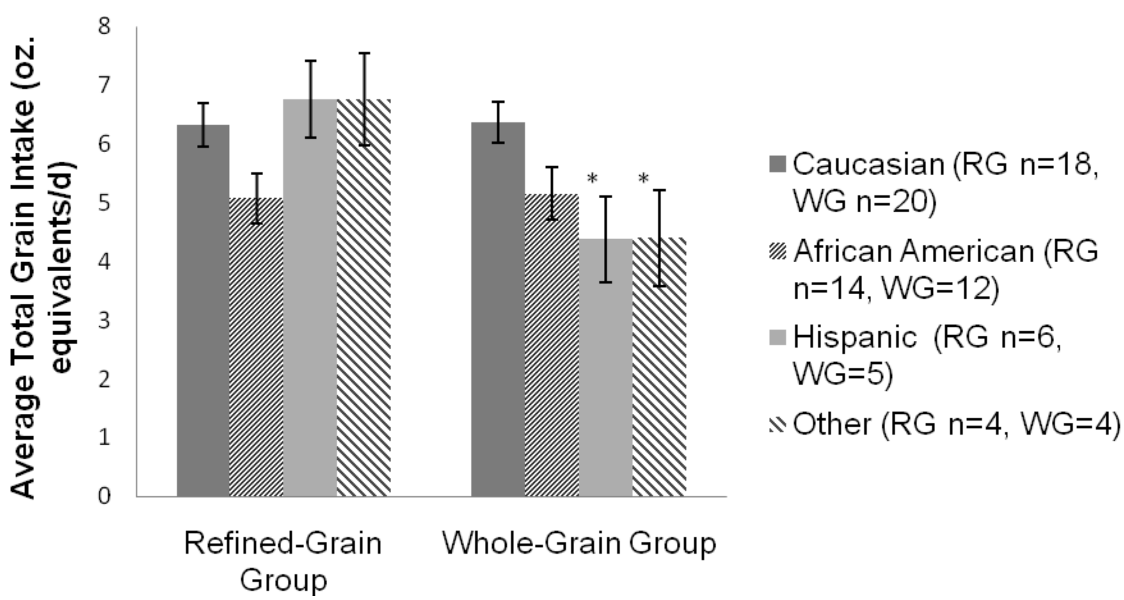

Figure 4-7. Total grain intake by treatment group across the pre-baseline and treatment periods by subjects of different races and ethnicities randomized to the refined- or whole-grain groups.

Table 4-5. Tests of effect slices for group and race/ethnicity interaction.

Race/ethnicity	Num DF	Den DF	F Value	Pr > F
Black/African American (n=26)	1	151	0.02	0.8997
Hispanic/Latino (n=11)	1	151	5.90	0.0163
Other (n=8)	1	151	4.40	0.0376
White (n=38)	1	151	0.01	0.9363

Table 4-6. Tests of effect slices for group and race/ethnicity interaction.

Group	Num DF	Den DF	F Value	Pr > F
Refined Grain (n=42)	3	151	2.74	0.0452
Whole Grain (n=41)	3	151	3.70	0.0132

Table 4-7. The main effects of gender, group, study period, race/ethnicity, and their interactions on average daily intake of refined grain.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Gender	1	151	8.18	0.0048
Group (RG, WG)	1	151	42.98	<.0001
Gender*Group	1	151	3.04	0.0833
When (pre-baseline, treatment)	1	151	0.17	0.6848
Gender*when	1	151	0.04	0.8502
Group*when	1	151	38.49	<.0001
Gender*Group*when	1	151	0.58	0.4473
Race/ethnicity	3	151	0.56	0.6425
Group* Race/ethnicity	3	151	1.59	0.1940

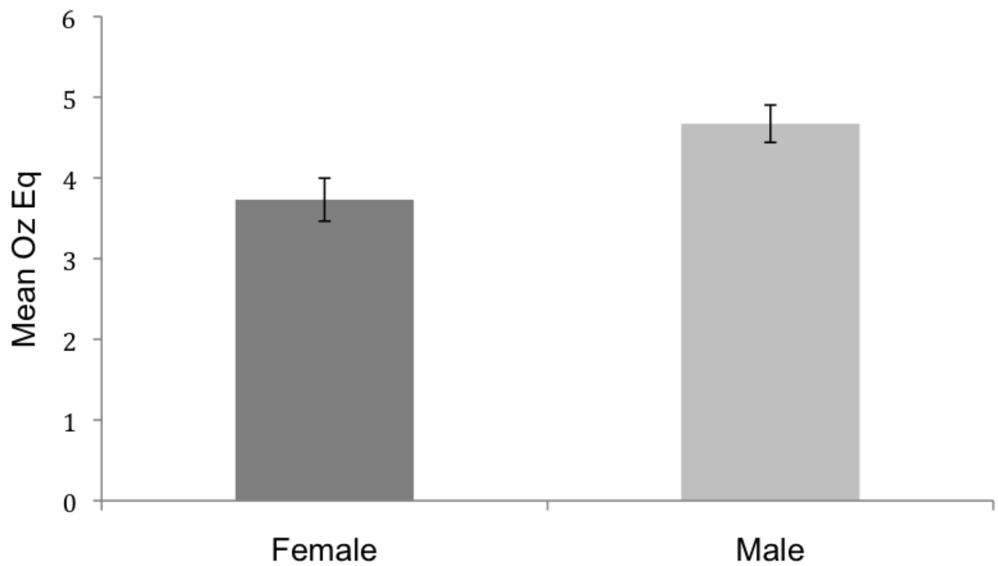

Figure 4-8. The least squares means for the average daily intake of refined grain (ounce equivalents) by females and males. The interaction between genders was significant (P= 0.0048).

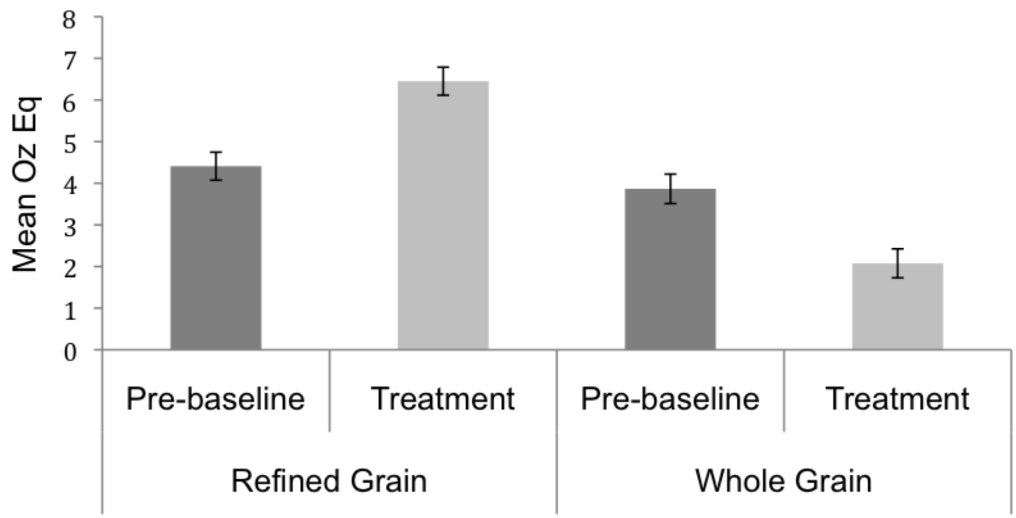

Figure 4-9. The least squares means for the average daily intake of refined grain by subjects in the refined-grain or whole-grain groups during the pre-baseline and treatment periods. The interaction between treatment group and study period was significant (P<0.0001).

Table 4-8. The main effects of gender, group, study period, race/ethnicity, and their interactions on average daily intake of whole grains.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Gender	1	151	0.35	0.5539
Group (RG, WG)	1	151	29.20	<.0001
Gender*Group	1	151	2.70	0.1023
When (pre-baseline, treatment)	1	151	23.38	<.0001
Gender*when	1	151	3.25	0.0734
Group*when	1	151	104.67	<.0001
Gender*Group*when	1	151	0.00	0.9688
Race/ethnicity	3	151	4.04	0.0085
Group*Race/ethnicity	3	151	2.44	0.0667

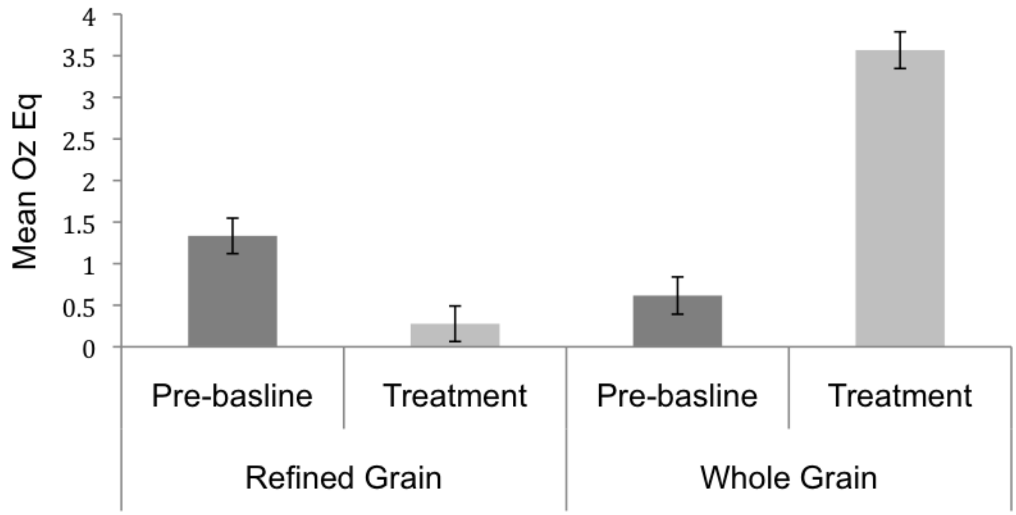

Figure 4-10. The least squares means for the average daily intake of whole grains (ounce equivalents) by all subjects in the refined-grain or whole-grain groups during the pre-baseline and treatment periods. The interaction between treatment group and study period was significant (P<0.0001).

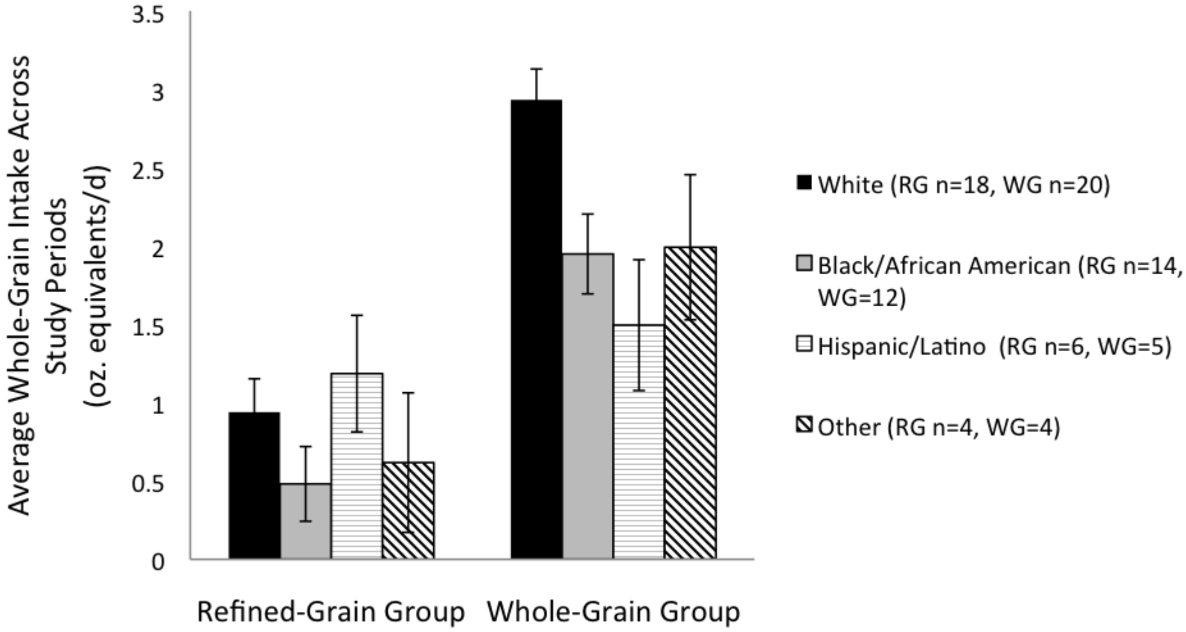

Figure 4-11. Whole-grain intake (ounce equivalents) by treatment group across the pre-baseline and treatment periods by subjects of different races and ethnicities.

Table 4-9. Tests of effect slices for group and race/ethnicity interaction.

Ethnicity	Num DF	Den DF	F Value	Pr > F
Black/African American	1	151	17.68	<.0001
Hispanic/Latino	1	151	0.31	0.5808
Other	1	151	4.58	0.0340
White	1	151	47.38	<.0001

Table 4-10. Tests of effect slices for group and race/ethnicity interaction.

Group	Num DF	Den DF	F Value	Pr > F
White	3	151	1.15	0.3305
Green	3	151	5.37	0.0015

Table 4-11. The main effects of gender, group, study period, race/ethnicity, and their interactions on average daily intake of fruit.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Gender	1	151	1.24	0.2665
Group (RG, WG)	1	151	0.84	0.3610
Gender*Group	1	151	5.88	0.0165
When (pre-baseline, treatment)	1	151	1.45	0.2305
Gender*when	1	151	1.23	0.2688
Group*when	1	151	0.02	0.8975
Gender*Group*when	1	151	0.71	0.4006
Race/ethnicity	3	151	0.64	0.5909
Group*Race/ethnicity	3	151	0.68	0.5662

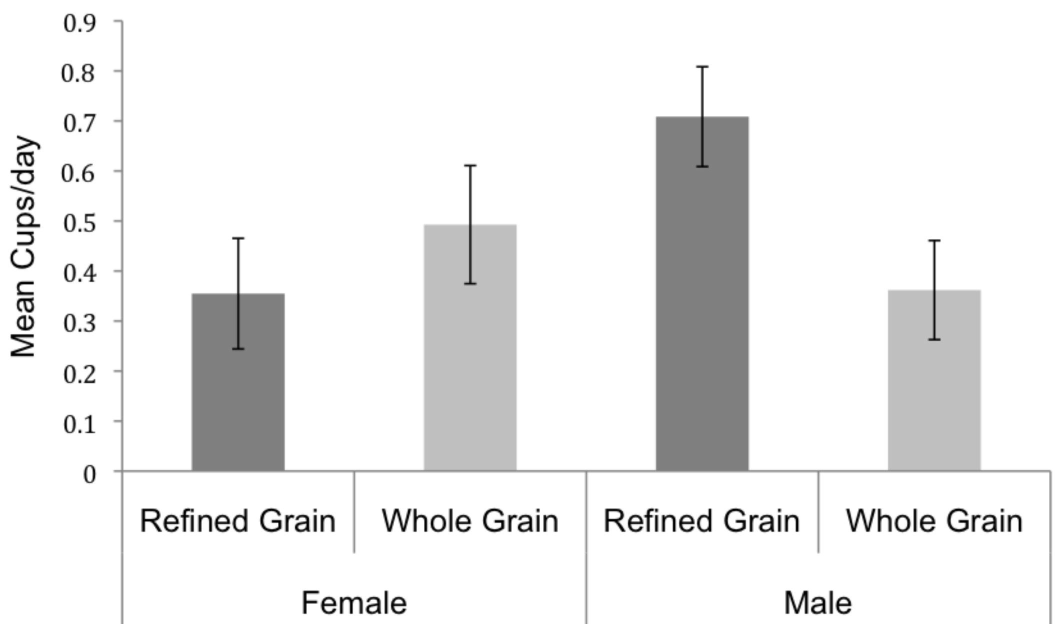

Figure 4-12. The least squares means for the average daily intake of fruit (cup equivalents) by gender and treatment group. The interaction between treatment group and gender on fruit intake was significant (P=0.0165).

Table 4-12. The main effects of gender, group (refined grain [RG] or whole grain [WG]), study period (pre-baseline or treatment), race/ethnicity and their interactions on average daily intake of vegetable (cup equivalents).

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Gender	1	151	4.95	0.0276
Group (RG, WG)	1	151	0.50	0.4818
Gender*Group	1	151	0.70	0.4042
When (pre-baseline, treatment)	1	151	1.42	0.2350
Gender*when	1	151	0.02	0.8908
Group*when	1	151	0.57	0.4511
Gender*Group*when	1	151	1.15	0.2860
Race/ethnicity	3	151	0.93	0.4299
Group*Race/ethnicity	3	151	0.40	0.7502

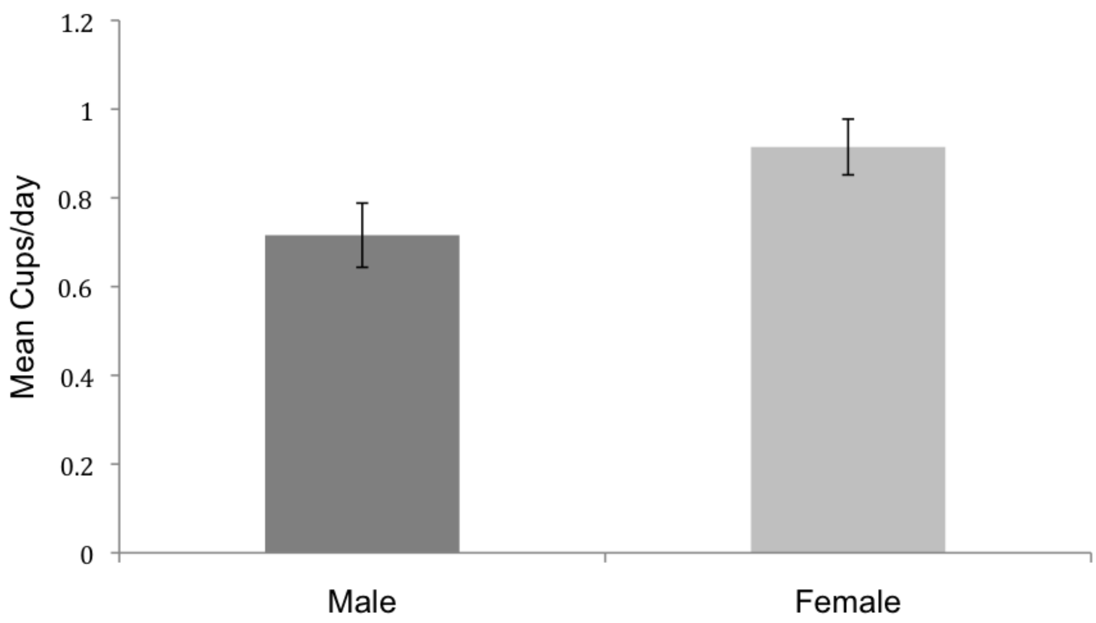

Figure 4-13. The least squares means for the average daily intake of vegetables (cup equivalents) by gender. The interaction between genders was significant. (P=0.0276).

CHAPTER 5
DISCUSSION AND CONCLUSION

This study provides new information on the effect of providing whole grain foods to adolescents. Previous work suggests that adolescents are not consuming the recommended amount of whole grains per day (64). In fact, most adolescents are consuming less than 1 serving of whole grains per day (65). In this study, middle school students (ages 11-16 years old) were randomized to receive weekly groceries of either refined-grain or whole-grain foods. Pre-baseline and treatment period 24-hour targeted recalls were obtained from all participants in order to report grain, fruit, and vegetable intake. At least two-thirds of the recalls were obtained on weekdays. The percentages of weekday recalls were similar across treatment groups, genders, racial and ethnic groups, and pre-baseline and treatment periods. Differences in dietary intake are not due to difference in dietary patterns observed between weekdays and weekends.

As anticipated, the average total grain intake, upon receiving the study foods, increased by 1-ounce equivalent when male and female intake was combined. The average total gain intake in males was over 6-ounce equivalents per day (recommended level is 6- to 7-ounce equivalents per day) and was greater than that of female subjects (Figure 4-4). The average total grain intake in females was over 5-ounce equivalents per day (recommended level is 5- to 6-ounce equivalents per day). Across the pre-baseline and treatment periods, participants in the refined-grain group consumed 1-ounce equivalent more total grains than participants in the whole-grain group (Figure 4-5). Hispanic and “Other” subjects took in approximately 2.5-ounce equivalents fewer total grains when they were randomized to the whole-grain group (Figure 4-7). In terms of diet improvement, the quality of the diet improved for both treatment groups, with an increase in total grain intake to the recommended amount (Figure 4-6).

The pre-baseline intake of whole grains was not different between groups and was similar to the typical intake for adolescents (1-ounce equivalent). When participants were provided with groceries of whole-grain foods and encouraged to eat at least 3 different study foods per day, their whole grain intake was over 3-ounce equivalents higher than participants receiving refined-grain foods (Figure 4-10). Participants in the whole-grain group increased their intake of whole grains by 3-ounce equivalents, whereas those in the refined-grain group decreased their intake of whole grains by a 1-ounce equivalent (Figure 4-10). In terms of ethnicity and race, Whites took in a 1-ounce equivalent more whole grain than any other racial/ethnic group across both study periods and groups (Figure 4-11). During pre-baseline and treatment periods, Whites and African Americans took in 2-ounce and 1.5-ounce equivalents more whole grains when they were in the whole-grain versus refined-grain group. Hispanic subjects took in an average of 1.3-ounce equivalents of whole grains and there was no significant difference in intake when randomized to the whole-grain group (Figure 4-11). Explanations for the poor intake of whole grains by Hispanic participants need to be pursued. Possible explanations include: language barrier when reporting food intake, unfamiliar study foods, and challenges in food delivery or pick up. According to the results, as a group, middle-school students will increase their intake to the recommended amount of whole grains if they are provided whole-grain foods.

All participants consumed on average, 4-ounce equivalents of refined grain during the pre-baseline period. The participants randomized to the whole-grain group decreased their intake of refined grains by almost 2-ounce equivalents (Figure 4-7). On average, males consumed 1-ounce equivalent more refined grains than females across the pre-baseline and treatment periods (Figure 4-8). As predicted, participants in the refined-grain group increased their intake of refined grains by 2-ounce equivalents per day (Figure 4-9). However, participants randomized to the whole-grain group still consumed 2-ounce equivalents of refined grain foods during the treatment period (Figure 4-9). This difference is attributed to the intake of males in the refined-grain group. There was no difference in refined grain intake among racial and ethnic groups. The results show that when middle school students are provided whole grain foods, they will decrease but not eliminate their refined grain intake. Also, when provided refined grains and encouraged to eat at least three different grain foods per day, middle-school students will increase their intake of refined grains.

Across ethnic and racial backgrounds and study period, fruit and vegetable intake was not significantly altered. The average daily intake of fruit was approximately one-half cup. The USDA recommends that girls consume 1.5 cups per day and boys consume 1.5 to 2 cups per day and so fruit intake was low for all subjects and not changed by treatment group. A significant difference was found between the gender and treatment group interaction for average daily fruit consumption (Figure 4-12). The average daily intake of vegetables was less than one cup. The USDA recommends that girls consume 2 to 2.5 cups per day and boys consume 2.5 to 3 cups per day and so vegetable intake was low for all subjects and not changed by treatment. A significant difference was found between the average daily intakes of vegetables across gender, showing that females consumed more vegetables than males (Figure 4-13).

Few studies have looked at ethnic influences on whole grain consumption in this age group. This study found that subjects who self-identified themselves as Hispanic/Latino did not have an increase in intake of whole grains when randomized to the whole-grain versus refined-grain group. Interestingly, the data suggest that these subjects did not eat the whole grain study foods or replace them in their diet with refined grains (i.e., intake of refined grains was not dependent upon race/ethnicity). Total grain intake across study periods was 6.7±0.7 versus 4.4±0.7 (*P*=0.016) in Hispanic/Latino subjects randomized to the refined-grain versus whole-grain groups, respectively. Due to the small sample size, we could not examine the three-way interaction between treatment group, study period (baseline, treatment), and race/ethnicity. Project EAT (Eating Among Teens) found differences in whole-grain intake according to race-ethnicity. Approximately 90% of the participants from the mixed/other ethnic group consumed 1 or less than 1 whole-grain servings per day. Results from the USDA's 1994-96 Continuing Survey of Food Intakes by Individuals show that only 5% of Hispanic adults consume greater than or equal to 3-servings of whole-grain per day. Participants most inclined to consume higher levels of whole grains were generally White and had higher education. The Hispanic group had the lowest whole grain intake when randomized to the whole grain group. This suggests that foods provided may have been unfamiliar; therefore ethnically acceptable options need to be explored when trying to increase whole grain consumption in ethnic populations.

This study reveals, for the first time, that when middle-school students are provided whole grain foods and encouraged to eat at least three different whole grain foods per day, their whole grain intake will increase to the daily-recommended amount (at least 3-servings per day). School meals represent a strategic point of intervention for children because 31 million children participate in the National School Lunch Program, therefore introducing whole grains in school lunch may be the key to increasing consumption to the recommended amount in adolescents (66). Not only have whole grains been associated with a reduced risk of certain diseases, consumption has also been related with improved diet quality and nutrient intake in children and adolescents (65). Currently, the USDA has issued a policy for encouraging adherence to the Dietary Guidelines for Americans in the National School Lunch Program (3). Future studies that involve an intervention with whole-grain foods should include a follow-up assessment to see if participants changed their whole grain consumption post-intervention.

APPENDIX A
DOCUMENTS SUBMITTED TO THE INSTITUTIONAL REVIEW BOARD

Protocol

1. Project Title

The addition of whole grains to the diets of middle-school children:
A study of digestive health and immune defenses

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Westwood Middle School (970 students: grades 6 through 8)
Preliminary verbal approvals of the basic study design and study location have been obtained from the Alachua County School District and Westwood Middle School. Final approvals are contingent on obtaining University of Florida Institutional Review Board approval.

3. Abstract:

Dietary Guidelines for Americans recommend that children and adolescents "consume whole-grain products often; at least half the grains should be whole grains"; however, no randomized, controlled studies, to our knowledge, examine the role of whole grains on gastrointestinal and immune health in adolescents. Therefore, the purpose of this study is to determine if adolescents eating diets rich in whole grains vs. diets rich in refined grains (i.e., a typical diet) have improved markers of digestive health and natural immune defenses. Middle-school students (11-15 years of age, n=80) will be recruited from Westwood Middle School, Gainesville, Florida, and randomized to receive $\geq$80 g of whole grains ($\geq$5 servings) or similar foods made with refined grains each day for six weeks. Based on treatment group, subjects will be provided either whole grain or refined grain foods and snacks. They will be instructed on how to use these foods to replace other foods already contained in the diet. Stool, blood, and saliva samples will be obtained at baseline and at study end to examine the microbiota and markers of digestive and immune health. Daily records will be maintained by the students to assess bowel habits and compliance. It is anticipated that whole grains will increase stool bulk resulting in increased stool frequency and softer stools. Additionally, fermentation of the fiber within the colon will alter the microbiota profile. Because the majority of the immune system resides within the gastrointestinal tract, improved balance of the intestinal microbiota may prime the immune system thus contributing to improved immune defense.

4. Background:

Dietary Guidelines for Americans recommend that children and adolescents "consume whole-grain products often; at least half the grains should be whole grains" (1). Whole grains are seeds that consist of the bran, germ, and endosperm. The bran is the outer cell wall of the grain seed which contains fiber, antioxidants, B vitamins and most of the minerals found in grains (e.g., iron, copper, zinc, magnesium). Ferulic acid is a phytochemical that links cellulose to other polysaccharide components within the cell wall. Ferulic acid also functions as an antioxidant. The germ is the part of the seed that sprouts into the new plant. It contains B vitamins, some protein, phytochemicals, antioxidants such as vitamin E, and unsaturated fats. The endosperm, which is the largest part of the grain, provides energy for the sprouting seed. It contains carbohydrate, B vitamins and minerals. Refined flours are made from the endosperm. During the refining process, the majority of the fiber, vitamins, minerals, antioxidants and phytochemicals are lost; however, the B vitamins, folic acid, and iron are added back to the refined flours.

Whole grains offer the benefit of all of the aforementioned nutrients and phytochemicals. Additionally, grains provide fiber, resistant starches, and oligosaccharides that contribute to fecal bulking and the production of short-chain fatty acids via bacterial fermentation. Epidemiological studies associate consumption of whole grains with protection from cancer, cardiovascular disease, diabetes, and obesity; however, few randomized, controlled studies examine the effect of whole grains on health (2-4). No randomized, controlled studies, to our knowledge, examine the role of whole grains on gastrointestinal and immune health in adolescents. Because whole grain foods are typically more expensive and possibly not as well accepted than the refined grain version, it is a challenge to add whole grain foods to school breakfasts and lunches within public schools. Demonstrating a benefit before recommendations for substituting half a day's intake of grains with whole grains are initiated seems to be a logical first step. Therefore, the purpose of this study is to determine if adolescents eating diets rich in whole grains vs. diets rich in refined grains (i.e., a typical diet) have improved markers of digestive health and natural immune defenses.

Middle-school students (11-15 years of age) will be recruited from Westwood Middle School and randomized to receive $\geq$80 g of whole grains ($\geq$5 servings) or similar foods made with refined grains each day for six weeks. According to MyPyramid Plans (www.mypryamid.gov), adolescent males require between 5 to 10 servings of grains per day with 3 to 5 of these servings coming from whole grains. Adolescent females require between 5 to 8 servings of grains per day with 3 to 4 servings from whole grains. Based on treatment group, subjects will be provided either whole grain or refined grain foods and snacks. They will be instructed on how to use these foods to replace other foods already contained in the diet. Stool, blood, and saliva samples will be obtained at baseline and at study end to examine the microbiota and markers of digestive and immune health. Daily records will be maintained by the students to assess bowel habits and compliance. This study will be completed during the early spring when students are undergoing their Florida Comprehensive Assessment Tests (FCAT). This is also peak flu season for this region. The academic stress associated with the exams and the increased prevalence of cold and flu may provide for a larger treatment difference in markers of immune health.

Anticipated Results

Based on work by Costabile et al.., in adults (20 to 42 years of age) fed 48 g of whole grain wheat per day, we anticipate seeing improved bowel habits and a change in the microbiota profile with an increase in fecal bifidobacteria and lactobacilli (2). Whole grain wheat is a source of antioxidants (ferulic acid). The fasting concentration of ferulic acid tripled in the plasma after subjects consumed a whole grain wheat diet for 3 wk. The authors suggested that regular consumption of whole grains results in a slow and continuous release of phenolic compounds, such as ferulic acid, into the bloodstream (2). In this regard, ferulic acid could be used as a biomarker for compliance for the adolescents consuming the whole-grain diet.

Continuous release of compounds with antioxidant potential could also improve antioxidant capacity in subjects. If this is the case, we would anticipate seeing an increase in the serum antioxidant capacity and a decrease in the inflammatory cytokines and acute phase proteins (i.e., C-reactive protein, fibrinogen). However, this assumes that acute phase proteins and inflammatory cytokines are elevated in adolescents. We would anticipate seeing elevated C-reactive protein, fibrinogen, and inflammatory cytokines in obese subjects, but this may not be the case in lean subjects (5).

Bacterial fermentation of fiber in the colon results in an increase in short-chain fatty acids, including butyrate the primary energy source for colon epithelial cells. We hope to see an increase in short-chain fatty acids in the stool samples with the whole grain diet; however, it would not be surprising if no differences in the short-chain fatty acid profiles were observed with the whole-grain intervention, as short-chain fatty acids are readily absorbed by the colon.

The academic stress model is associated with decreased natural killer cell activity, decreased mitogen (PHA/LPS) stimulated peripheral blood mononuclear secretion of interleukin (IL)-2, IL-4, and IL-1β, and increased days of cold and flu (6, 7). The stress associated with FCAT exams during cold and flu season may result in impaired immune defenses and increased cold and flu resulting in increased student absences. If improved health of the intestinal environment reduces the burden on the immune system, we would anticipate observing increased natural killer cell activity and decreased absences from school in subjects consuming the whole-grain diet.

Increased production of short-chain fatty acids and the release of phenolic compounds (i.e., potential antioxidants) in the whole grains by the intestinal microbiota may be responsible for improved immune health. In fact, an increase in bifidobacteria and lactobacilli is associated with increased blood natural killer cell activity and an increase in bifidobacteria is associated with increased fecal levels of secretory immunoglobulin A (sIgA) (8, 9). Because the colon is part of the common mucosal immune system, it is anticipated that an increase in fecal sIgA would also result in an increase in salivary sIgA. Altered microbiota and increased short-chain fatty acids and antioxidant compounds within the colon may also result in a decrease in colonic inflammatory cytokines. If this is the case, we would anticipate seeing a decrease in IL-17A and TNF-α within the fecal water. A shift in inflammatory status may also result in an increase in the epithelial cell-derived cytokines (i.e., IL-25 and IL-33) which promote Th2-dependent immune responses (9, 10).

Treatments that modulate the microbiota profile also modulate cytokine responses in mitogen-stimulated peripheral blood mononuclear cells. Vulevic et al. supplemented the diets of elders with a trans-galactooligosaccharide mixture and observed an increase in the numbers of beneficial bacteria including bifidobacteria and lactobacilli. The investigators also noted an increase in LPS-stimulated peripheral blood mononuclear cell production of the immunosuppressive cytokine IL-10 and a decrease in the proinflammatory cytokines IL-1β, TNF-α, and IL-6 (9). We anticipate altered fecal microbiota profiles with the addition of whole grains to the diets of adolescents. If this affects inflammatory status, we would be more likely to see this change following mitogen (LPS) stimulation of peripheral blood mononuclear cells versus finding measurable changes in serum levels. This is primarily because adolescents, especially lean teens, should not have elevated levels of inflammatory cytokines as is observed with advanced age and obesity. It is also more likely that the whole grain diet is associated with changes in Th1-Th2 cytokine balance rather than changes in T-cell subtypes. Therefore, we are proposing to measure Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-5, IL-10) cytokines in mitogen (PHA)-stimulated lymphocytes.

5. Specific Aims:

I. Examine the effect of incorporating five or more servings, with a minimum of three servings, of whole grains into the usual diets of adolescents on digestive health. The working hypothesis is that fiber in the whole grains will increase stool bulk resulting in increased stool frequency and softer stools. Additionally, fermentation of the fiber within the colon will alter the microbiota profile and increase fermentation products within the stool.

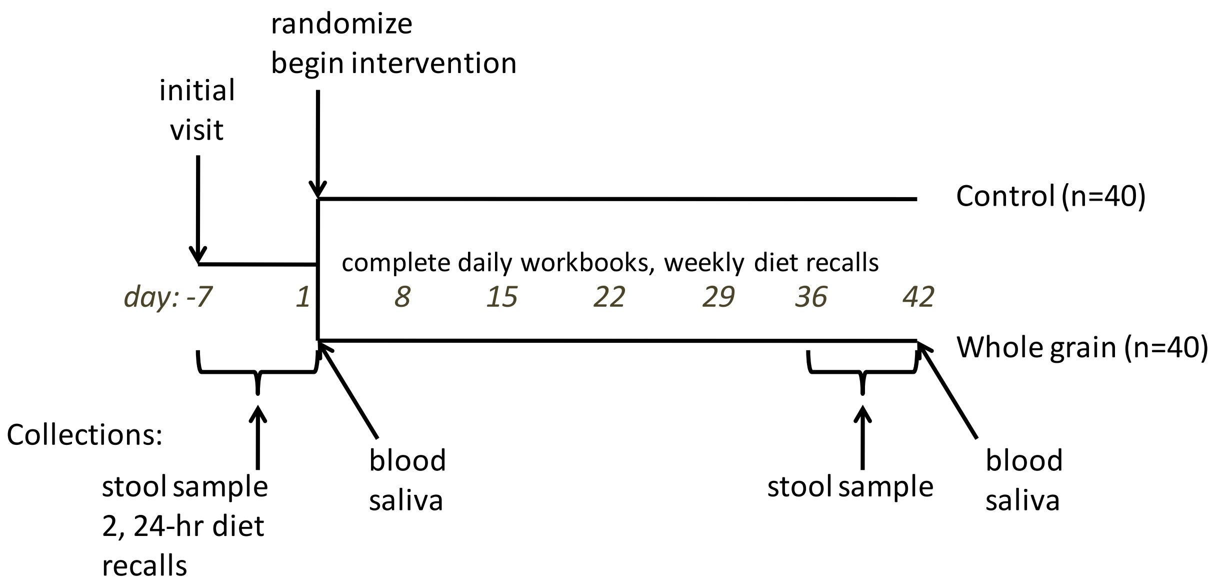
II. Characterize the effect of incorporating five or more servings, with a minimum of three servings, of whole grains into the usual diets of adolescents on immune defenses. The working hypothesis is that the whole grains will provide nutrients and phytochemicals important in natural immune defenses. Additionally, because the majority of the immune system resides within the gastrointestinal tract, improved balance of the intestinal environment (i.e., a microbiota profile associated with improved health) will prime the immune system thus contributing to improved immune defense.

Research Plan:

Study Design

A six week randomized, controlled, parallel arm study will be carried out with 80 healthy adolescents, ages 11-15 years enrolled in Westwood Middle School, Gainesville, FL (Figure 1). The study population will consist of healthy, middle-school students (primarily sixth and seventh graders) undergoing the Florida Comprehensive Assessment Tests (FCAT). These exams are typically given in February and early March, a period of time that coincides with peak cold and flu season in the state of Florida. Although only healthy students will be enrolled, the psychological stress associated with the FCATs may increase the incidence of cold and flu symptoms; thereby maximizing the differences in immune defense between treatment groups.

Institutional Review Board approval will be sought from the University of Florida IRB-1. Approval will also be sought from the Alachua County Public Schools and Westwood Middle School. Informed consent from one parent (as per UF guidelines for minimal risk interventions with minors) and assent from all study participants will be sought.



<u>Pre-baseline</u>
After obtaining informed consent and assent, the inclusion/exclusion criteria will be reviewed. Exclusion criteria will include: known gastrointestinal disease; other chronic disease; allergy to wheat, gluten, milk, egg, soy, nuts or any other foods or food ingredients; use of gastrointestinal motility agents or laxatives or immunosuppressive drugs or treatments; regular consumption of probiotics or yogurt (>3 servings per week), use of antibiotics in the four weeks prior to randomization, or refusing to provide a baseline blood or saliva sample. Potential subjects will be asked to identify all of the types of foods that they would be willing to eat as a participant. Subjects will only be included in the study if they are willing to consume at least three different foods on the list of foods (≥80 g of whole grains), every day for the six-week study. Eligible subjects and their guardian will then be instructed on how to obtain a stool sample and where to deliver the sample or call for pickup. Appointment times will be given for stool sample collection (estimated day/time), two 24-hour diet recalls, food delivery, and study start date. All participants will be given a sleeve of disposable soup-sized (i.e., larger sized) bowls. Subjects will be told to use these bowls to eat their cereal, pasta, and rice. These same bowls will then be used during the targeted 24-hour diet recalls to more accurately describe serving sizes of grain products. Contact information will be obtained from the subject and family. The subject or family will be contacted via phone or email to remind them of study appointments, schedule new appointments, discuss study procedures, deliver study foods, obtain the 24-hour diet and activity recalls, etc. We will also take a picture of the student to keep in his subject file for identification purposes.

In the week before randomization a single stool sample will be obtained from each subject. If subjects decide that they cannot provide the stool sample after assenting, they will not be dropped from the study as long as we have n=25 per group (50 total) willing to provide the sample. This number should be sufficient to observe significant differences in microbiota between study groups. A 24-hour activity recall will be obtained to broadly classify levels of activity (e.g., sedentary, moderately active, vigorously active). Activity recalls will provide import information because activity can influence both bowel and immune function. Height, weight, age, and body mass index will be obtained.

Trained study coordinators or graduate dietetic students will contact study participants on two different days the week or so before randomization to administer a targeted 24-hour diet recall. Subjects will be asked to list all of the foods and beverages they ate in the previous 24 hours (midnight to midnight); but specific information (i.e., serving size, whole grain vs. refined grain, method of preparation, etc.) will only be obtained for grain products, fruits, and vegetables. Food models, measuring cups and spoons, and the disposable soup bowls will be used to help determine serving size. Parents or guardians may be contacted to obtain additional information or clarification of dietary intake or activity during the recall period. It is anticipated that many of the subjects will be participating in the school breakfast and lunch program at the middle school. At present, the only whole grain foods on Alachua County Schools' menu are whole grain pancakes and tortillas. The investigators will work with closely with the food service director at the middle school and the dietitian with Alachua County Public Schools to obtain menus, recipes, and lists of whole grain foods provided during the study period. This will help with the accuracy of the targeted 24-hour diet recalls.

Baseline

On the first day of the study, after an overnight (6-hour minimum) fast, subjects will have their blood drawn by a licensed phlebotomist in the school clinic. Approximately a tablespoon of blood will be obtained to assess immune health (see outcomes below). Saliva will be obtained by unstimulated, passive drool. Students will be asked to rinse their mouth thoroughly with water 10 minutes before the sample is collected. They will then be asked to spit all of their saliva into a collection tube for a defined period, such as two to three minutes. Students who refuse to provide a blood or saliva sample will not be eligible for randomization into the study.

Randomization. Because obesity is associated with increased inflammation even in adolescents, subjects will be stratified based on BMI ($<95^{th}$ BMI percentile for age or BMI <30, $\geq 95^{th}$ BMI percentile for age or BMI ≥ 30) and participants will be randomized to two groups, a whole grain or refined grain group. This study will not be blinded but study groups will be denoted as something other than "whole or refined grain" to de-emphasize the differences between the groups.

Intervention. Foods, such as pasta, rice, bread, cereal, and pancake mix, will be provided for the study subject's family for the six-week study period. Individual servings of foods, such as breakfast bars, cereal, or snack foods, will be provided to the subject either in their family food package or at a study booth at the school. As much as possible, food packages for the subject and family will be prepared based on each subject's food preferences. Where possible, package labels on the food will be removed. Study staff will be available at the school (e.g., before and after school or during home room or lunch) to distribute study foods and assist study participants with questionnaire completion. Study participants will be given the opportunity to consume study foods at school. Students will be asked to consume at least three different study foods each day. It is anticipated that the students will consume more than one serving of each type of food. There is no maximum limit for daily study food consumption. Ideally, we would like to see students eat five servings (80 g of whole grain) per day. However, we will discourage overeating and weight gain. Weekly body weights will be obtained.

Daily and Weekly Measures

Subjects will be given study workbooks with tear-out pages for daily logs. Students will be asked to come to the study booth each school day to drop off their completed logs. The daily logs will ask about the number of stools, consumption of study foods, level of stress, and gastrointestinal symptoms such as gas, bloating, and abdominal discomfort. To avoid giving the subjects the idea that they should be stressed and have gastrointestinal symptoms, we will embed these questions with other more positive distracting questions, such as level of happiness, etc. Each week the subject will meet with a study coordinator or graduate dietetic student to be weighed (weights will be done in kg) and to provide a targeted 24-hour diet recall. If the subject is unable to complete the study record or requires help, a study coordinator with very limited knowledge of the study design and no knowledge of the study group to which the subject is assigned, will contact the subject daily and record the answers to the questions. This will be done to eliminate any bias when recording answers for the subject.

Final Study Week

During the last week of the study intervention, one stool sample and 24-hour targeted diet and activity recalls will be obtained. On the last day of the study ($\pm$3 days), blood and saliva collections will be obtained as described for baseline collections. Height and weight will also be reassessed and the subjects will be asked about any sicknesses during the study. Information on new medications, antibiotic use, and influenza vaccinations will be obtained at that time. Parents, guardians, or physicians (for severe adverse events) may be contacted to help clarify subject responses. Absences from school will be recorded by the study staff with the help of the school attendance office over the course of the study. Students will be asked if they missed school due to illness and if so what type of illness.

Incentives

In addition to study foods, subjects will receive coupons with a total of seven coupons possible: one coupon for completing the pre-study week and six additional coupons for completing each of the six study weeks. Each week they participate they will receive another coupon. At the end of the study they can trade the coupons for ONE prize. Subjects may select a prize from a category or level for which they qualified for or from a level worth fewer coupons. There were be seven levels of prizes that represent an increase in dollar value of about $14 each or approximately $100 for seven coupons (i.e., completing the study). Rewards are of reasonable value to prevent the possibility of coercion. In a recently published article, middle-school students received a $10 bookstore gift card for providing height, weight, one diet recall, and one interview (11).

Outcomes (Note: some samples may be sent off campus for analyses. The only identifying information that will be included with the sample will be subject number and date – no PHI.)

- ***Digestive Health:***
  - **Bowel Habit:** Bowel movement frequency and GI symptoms (e.g., gas, bloating, and abdominal discomfort) will be recorded daily by subjects.

- **Stool samples:** Consistency of collected stool samples will be scored by study staff using the Bristol Stool Form Scale. Stools will be weighed and pH will be obtained.
  - **Fermentation profiles:** Short-chain and branched-chain fatty acids, lactate, and ammonia will be quantified in stool samples.
  - **Microbiota studies:**
    - Changes in overall microbiota diversity
    - Quantitative changes in the proportions of bacteria, such as Bifidobacteria, Lactobacilli, C. perfringens and E. Coli, will be determined to give an indication of the prebiotic effect of the interventions.

- ***Immune Health:*** Approximately one tablespoon of blood will be obtained for the lymphocyte, natural killer cell, and serum studies described below. Ideally, this should be a fasting blood draw (6-hour minimum fast).
  - Peripheral blood mononuclear cells (PBMC):
    - **mitogen-induced lymphocyte proliferation** (mitogens: PHA, ConA, Pokeweed, and LPS)
    - **mitogen-induced cytokine production** (mitogens: PHA and LPS) cytokines: such as but not limited to Th1 [IL-2, IFN-γ], Th2 [IL-4, IL-5, IL-10], inflammatory [IL-1β, IL-6, TNF-α] and misc. [IL-12p40, IL-12p70, IL-8, IL-17, TGF-β]
    - **natural killer cell activity**
  - Serum
    - **antioxidant capacity**
      - ABTS (plus triglycerides)
      - phenolic compounds present in whole grains, such as ferulic acid (the main phenolic compound in whole wheat, which is often used as a biomarker of cereal grain ingestion)
    - **acute phase proteins and inflammatory cytokines** (C reactive protein, fibrinogen, IL1β, TNF-α, IL-8, IL-6)

  - Fecal water:
    - **sIgA**
    - **cytokines**: such as IL-25 and IL-33 (epithelial cell-derived cytokines that enhances Th2-dependent immune responses), IL-17 (proinflammatory), IL-10 (immunosuppressive), TNF-α (proinflammatory)
    - **pH**

  - Saliva
    - **sIgA**

- ***Well-being:***
  - Level of daily stress (0 to 10)
  - Sick days, school days missed, doctor visits, new medications

- ***Other information:***

- Height, weight, body mass index (BMI), BMI percentile for age
- Age, gender
- Dietary intake: 8-targeted 24-hour diet recalls, daily intake of study foods
- Level of activity: two - 24-hour activity recalls (one at baseline and one during the final week). This will be basic information to classify the subjects as sedentary, moderately active, or vigorously active.

Sample Size

Based on changes in fecal water IgA with whole-grain associated increases in bifidobacteria, we estimate that 80 subjects will be required to detect a change in IgA of 2.0 mg/g feces with 80% power, $\alpha<0.05$, and 20% attrition rate (2, 8). Only one child from each household will be enrolled to avoid having two children from the same household being randomized to two different treatment groups. Sixth and seventh graders will be recruited and enrolled first. If we do not have sufficient numbers of sixth and seventh graders, then eighth graders will be enrolled. At randomization, students will be stratified by BMI (<95th BMI percentile for age or BMI <30, $\geq$95th BMI percentile for age or BMI $\geq$30). Data will be analyzed two different ways – compliant and intent to treat. Compliant subjects will be those who average at least 3 servings (48 g) of whole grains per day (whole grain group) or less than one serving (16 g) of whole grains per day (control group) based on the average intake from the weekly targeted 24-hour diet recalls that were completed during the intervention. Stool microbiota data from subjects who begin a course of antibiotics during the study will not be included in either group.

Extension Activity

In appreciation to Westwood Middle School's participation in the study, a school extension intervention will be carried out at the completion of the study. Teachers at Westwood will have access to whole grain and fiber lesson plans (on a CD) and classroom resources. They will also have the option to have one of the nutrition student study assistants give a presentation in their classroom on understanding whole grains and fiber. Educational materials will also be made available to the parents of all subjects and the Food and Nutrition Services of Alachua County Public Schools.

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Tentative Time Line (Dates are subject to change.)

April 2009	Meet with school officials to discuss study protocol.
May 2009	Sign contract. Write IRB proposal.
June 2009	Investigators meet to discuss specifics of the protocol.
August 28, 2009	Paperwork submitted to UF IRB for approval.
September 2009	• Approved UF IRB paperwork submitted to Alachua County Public Schools for approval. • Standard operating procedures for immune function studies completed.
September 2009	• (If possible) Present study to parents at Westwood open house. • Contact information collected from interested students/parents.
November 2009	• Study staff trained and recruits full time. • Laboratory staff trained.
February 1-5, 2010	Initial visit: stool collection and 24-hr recalls for first group of students
February 8-11, 2010	• Randomize first group of students (Flex day – Friday, Feb. 12) • Initial visit, stool collection 24-hr recalls for second group of students
February 16-19, 2010	Randomize second group of students (Holiday – Monday, Feb 15)
March 8-12, 2010	FCAT exams, UF spring break
March 15-19, 2010	Stool collection and 24-hr recalls for first group of students
March 22-26, 2010	• Final visit blood draw first group • Stool collection and 24-hr recalls for second group
March 30-April 2,	Final visit blood draw second group

2010	
April 2010	Begin immune function and fecal studies/assays. Begin data entry.
September 2010	Begin manuscript preparation.
May 1, 2011	Contract end.

7. Possible Discomforts and Risks:

- Typical risks and discomforts associated with drawing blood. We will minimize any discomforts by using an experienced phlebotomist.
- Some students may feel sick if they cannot eat first thing in the morning before the blood draw. After the students have their blood drawn, they will be given a continental breakfast, containing appropriate study foods based on their study group (baseline draw). At the final blood draw, students will be allowed to choose from a variety of breakfast items.
- Some students may be uncomfortable providing the stool samples. This will be minimized by giving the students/parents the option of bringing fresh stool to study staff at the school in the morning or paging study staff to pick up the samples from their home. Students who will be providing samples at school will be told to get a pass to go to the school clinic. Stool collection materials will be available in the clinic and study staff will be available to take the samples. Students providing fresh samples in the evening or on weekends will be asked to page the study staff for pick up.
- Some students may be uncomfortable describing their bowel habits. To hopefully make the students more comfortable with this and all study procedures, every attempt will be made to pair subjects up with the same study coordinator for the entire study.
- Students receiving the whole grain foods may experience gas, bloating, or abdominal discomfort from the fiber in the grains. Students experiencing these problems may need to decrease their intake of the whole grains and then slowly increase intake as their gastrointestinal tract begins to adjust to the extra fiber.
- Some students may gain or lose weight while participating in the study. This may or may not be a desirable outcome. We hope to minimize this by instructing students to use the foods to replace other foods in their diets. We will also monitor student weight over the course of the study.
- Some student may be uncomfortable having their height and weight measured. To minimize this concern, weights will be obtained in a private location. Additionally, weight and height measurements will be obtained and recorded in kg and cm, units that are less familiar to students.

8. Possible Benefits:

Westwood Middle School has a high percentage of students qualifying for free and reduced lunch and therefore may not have access to nutritious foods at home. Foods provided to the family and the student would provide nutrients and energy to all participants. The students randomized to the whole grain group will be receiving fiber and additional phytonutrients / antioxidants that may improve gastrointestinal and immune health. All students will be interacting with University of Florida study coordinators. It is possible that both groups may be more likely to attend classes because they enjoy interacting with their study coordinator and/or they are healthier because they are eating healthier foods.

9. Conflict of Interest:

The study investigators, who are employees of the University of Florida, are receiving funding from General Mills Bell Institute of Health and Nutrition, Minneapolis, MN to complete this study. Their careers may benefit if the results of this study are presented at scientific meetings or in scientific journals. The study investigators will receive no financial compensation from the completion of this study other than that provided as salaries by the University of Florida. The study sponsor will reimburse the researcher for study operating costs only. The study protocol was developed in collaboration with employees of General Mills and Europe-Cereal Partners Worldwide. Some of the study foods are being provided by General Mills and Europe-Cereal Partners Worldwide; however, where possible food package labels will be removed to avoid a potential conflict of interest. Most of the study foods used in this study will be purchased from area grocery or wholesale stores.

Recruitment Materials

- Script for Westwood Middle School Open House or other oral announcements/presentations to parents (PTA, etc.)
- Script for Westwood Middle School morning announcements
- Telephone / Initial Contact Script
- Flyers
- Email

Data Collection Forms

- Contact information
- Food preference list
- Inclusion/exclusion
- Initial visit
- Daily workbook questions
- Final visit
- Other information
- Stool Collection Information
- Targeted 24-hour diet recall
- 24-hour activity recall

Script for Westwood Middle School Open House or other oral announcements/presentations to parents (PTA, etc.)

Hello! My name is ________. I am a University of Florida study coordinator/principal investigator for the Food for Fun study at Westwood Middle School. We hope to enroll 80 Westwood students in February to examine whether whole grain foods are important for maintaining digestive health and immune defenses in adolescents.

The Dietary Guidelines for Americans recommend that adolescents consume half their grain foods as whole grains, but very few do this. Grain foods are breakfast cereals, breads, noodles, rice, crackers, etc. Whole grain foods are these grain products that contain all parts of the grain (i.e., bran, flour, germ). No studies show that the whole grain foods are better for adolescents. That is what we would like to determine with this study.

If your child participates in the study, we will give your family and primarily your child grain products to eat for 6 weeks. Foods will include breakfast cereals, bread, noodles, rice, and snack foods. We will monitor your child's digestive and immune health. To do this we will keep records of food intake and bowel movements and measure various parameters in your child's blood, stool, and saliva. We will ask for 2 stool samples and 2 blood draws. As a reward for completing all of the study procedures, your child will be given coupons to trade for rewards valued up to $100 for completing the study. These rewards will include things such as sports equipment, electronics, games, cameras, books, and bikes.

If you are interesting learning more, please contact…

Script for Westwood Middle School morning announcements

Good morning! We are looking for 80 Westwood students to help us with our Food for Fun study. We are trying to determine whether whole grain foods are important for maintain health in middle-school students. If you participate, you will receive a six-week supply of grain products for you and your family. Examples of these foods include breakfast cereal, bread, noodles, rice, and snacks. If you are interested in helping us out, please come to our Food for Fun booth (location/time/day) to get a study flyer.

Telephone / Initial Contact Script

Hello! My name is ________. With whom am I speaking? ___*(record name on telephone inclusion criteria form)*___ I would like to thank you for responding to the flyers regarding this study. I am a University of Florida study coordinator/principal investigator for the food study at Westwood Middle School. Do you have a few minutes right now for me to tell you about the study or would you like me to call back at a more convenient time? *(If yes continue, if no get contact information and good time to call.)*

First I will tell you about the study. If you think your child may be interested in participating I will then list the inclusion criteria so you can determine if your child qualifies (qualify) for this study. If you think (your child) qualifies, then I will set up the first study visit.

The Dietary Guidelines for Americans recommend that adolescents consume half their grain foods as whole grains. Grain foods are breakfast cereals, breads, noodles, rice, crackers, etc. Whole grain foods are these grain products that contain all parts of the grain (i.e., bran, flour, germ) not just the grain flour. No studies show that the whole grain foods are better for adolescents. That is what we would like to determine with this study.

If your child participates in the study, we will give your family and primarily your child grain products to eat for 6 weeks. Foods will include breakfast cereals, bread, noodles, rice, and snack foods. We will monitor your child's digestive and immune health. To do this we will keep records of food intake, bowel movements, and measure various parameters in your child's blood, stool, and saliva. We will ask for 2 stool samples and 2 blood draws. As a reward for completing all of the study procedures, your child will be given coupons to trade for rewards valued up to $100 for completing the study. These rewards will include things such as sports equipment, electronics, games, cameras, books, and bikes.

This study will start in early February and end six weeks later.

I will list off the inclusion criteria. Please think about these criteria, but do not answer. I will ask you at the end if your child still wants to participate in the study. If you think your child may qualify and still be interested we will need to sign an informed consent. At that time, I will again ask these questions to make sure your child qualifies. Stop me at any time if you have any questions.

Complete the Telephone / Initial Contact Script - Inclusion Criteria form.

Telephone / Initial Contact Script - Inclusion Criteria form.

Date:________________________________Time:_____________

Caller/Contact's name:____

Relationship to potential subject:____

Potential subject's name:____grade:______________

Call Back Number/ Cell Number: ____

E-mail:____

(Don't have them answer after each criterion.)

To participate in the study your child must

- be student at Westwood Middle School.
- be able to obtain parental/guardian consent.
- be willing to eat 3 different study foods each day for 6 weeks.
- be willing to provide 2 blood samples, 2 saliva samples, and 2 stool samples over the course of the study.
- NOT be taking medications for constipation or diarrhea.
- NOT be taking any antibiotics in the 4 weeks before randomization.
- NOT have any diseases or illnesses such as gastrointestinal disease (gastric ulcers, Crohn's, ulcerative colitis, etc.), other chronic diseases (diabetes, kidney disease, etc.) or immune-modulating diseases (HIV, AIDS, autoimmune, hepatitis, cancer etc.).

- Not have any food allergies (wheat, soy, egg, milk, gluten, nuts, or any other food or food ingredient)?

Is your child interested in participating?	Would the student be willing to provide 2 blood samples, 2 saliva samples, and 2 stool samples over the course of the study?	Best time to contact?	Appointment time?
☐ yes ☐ no	☐ yes ☐ no	Time:____________ Day:____________	Time:______________ Day:_______________

Food for Fun!

Join our food study.

80 healthy Westwood Middle School students are needed for a study examining the benefits of whole grain foods on digestive and immune health.

Study participants will receive a six-week supply of grain products (cereal, bread, noodles, rice, snacks). The study will begin February 1, 2010.

If you are interested, please contact:

Dr. Bobbi Henken

University of Florida

Phone: 352-682-4883

Email: nutrition-study@ufl.edu

Food for Fun!

Join our food study.

80 healthy Westwood Middle School students are needed for a study examining the benefits of whole grain foods on digestive and immune health.

Study participants will receive a six-week supply of grain products (cereal, bread, noodles, rice, snacks). The study will begin February 1, 2010.

If interested, please stop by our study booth and meet with our University of Florida Study Staff.

Where:

When:

Email:

To:

From:

Subject: Participants Needed for Food Study

Food for Fun!
Join our Food Study.

80 healthy Westwood Middle School students are needed for a study examining the benefits of whole grain foods on digestive and immune health.

Study participants will receive a six-week supply of grain products (breakfast cereal, bread, noodles, rice, snacks). The study will begin February 1, 2010.

If you are interested, please stop by our study booth
Where:
When:

Or contact:
Dr. Bobbi Henken
University of Florida
Phone: 352-682-4883
Email: nutrition-study@ufl.edu

Contact Information

Student name:____

Grade: 6th 7th 8th

Parent name:____

home phone:_______________________cell phone:__________________email:________________

address:____

Parent name:____

home phone:_______________________cell phone:__________________email:________________

address:____

Guardian name:____

home phone:_______________________cell phone:__________________email:________________

address:____

Advocate for Wards of State or other agencies (we would ask someone (teacher) not related to study)

____

If the legally authorized representative is consenting, document steps to determine validity of the proxy.

____

Homeroom teacher name:_____________________________________room #:____________________

Grade:____

Most likely address for stool sample pickup:____

Availability:

Time arrives at school?__________________________How? (walks, bus, parent):______________________

Time leaves school?______________________________How? (walks, bus, parent):______________________

Date:__________________________ **Subject Number:_____________**

Food Preference List

Please put a check mark in the box that best describes your likes and dislikes for each food.

Food	**I would eat it each day.**	**I would eat it a couple times a week.**	**I would never eat it.**
Granola bar			
Cereal bar			
Cereal that isn't sugared with milk			
Cereal eaten dry as a snack			
Oatmeal other hot cereals			
Bagels			
Bread slices as toast or a sandwich			
Tortillas			
Corn chips / other chips			

How many days a week do you eat the school-prepared lunch?____

How many days a week do you eat the school-prepared breakfast?____

Date:__________________________Time:____________ Subject Number:___________

Inclusion / Exclusion Criteria after Obtaining Consent and Assent

(Study coordinators to complete this form with the help of the child and guardian)

☐ **YES** ☐ No Parental/guardian consent has been obtained. Advocate appointed if necessary.

☐ **YES** ☐ No Student assent has been obtained.

☐ **YES** ☐ No Would you be willing to eat three different study foods each day for 6 weeks? (Check food preference list. Can they get in ≥80 g of whole grains per day)

☐ **YES** ☐ No Are you willing to provide 2 blood samples, 2 saliva samples, and 2 stool samples over the course of the study?

PLEASE NOTE THAT "YES" AND "NO" RESPONSES ARE REORDERED BELOW.

☐ **NO** ☐ YES Are you taking medications for constipation or diarrhea?

☐ **NO** ☐ YES Have you taken antibiotics recently (i.e., in the 4 weeks before randomization)?

☐ **NO** ☐ YES Do you take probiotics or consume >3 servings of yogurt per week?

☐ **NO** ☐ Yes Do you have any diseases or illnesses such as gastrointestinal disease (gastric ulcers, Crohn's, ulcerative colitis, etc.), other chronic diseases (diabetes, kidney disease, etc.) or immune-modulating diseases (HIV, AIDS, autoimmune, hepatitis, cancer etc.).

☐ **NO** ☐ Yes Do you have any food allergies (wheat, soy, egg, milk, gluten, nuts, or any other food or food ingredient)?

Pre-Qualifies? (Yes, if all boxes in first column marked)	☐ **yes**	☐ **no**

Qualifies? (Yes, if student provides a baseline blood sample and a saliva sample) ☐ **yes** ☐ **no**	Student provided a baseline blood sample Student provided a baseline saliva sample	☐ yes ☐ yes	☐ no ☐ no

Date:____________________________ **Subject Number:**__________

Initial Visit Data Collection Sheet

Age:__________________years **Date of Birth:** ________________________

Gender (circle): male female

Height:_______________cm **BMI:**______________________________________kg/M^2

Weight:_______________kg **BMI percentile:**_________________________

Ethnicity/Race:

________White ________American Indian/Alaskan Native

________Black/African American ________Native Hawaiian/Pacific Islander

________Hispanic/Latino ________Asian

Day 1 - Date:__________ **Subject Number:________**

Daily Workbook Questions

For each question below, circle the appropriate number.

1. Were you bothered by **stomach ache or pain** yesterday? Stomach ache refers to all kinds of aches or pains in your stomach or belly.

No stomach pain **Extreme pain**

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10

2. Did your **stomach feel bloated** yesterday? Feeling bloated refers to swelling in the stomach or belly.

No bloating **Extremely bloated**

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10

3. Were you bothered by **passing gas or flatus** yesterday? Passing gas refers to the release of air or gas from the bowels.

No gas **Very severe gas**

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10

4. What was your level of stress yesterday? Other ways to describe stress include being up-tight, anxious, worried, uneasy or stressed-out.

No stress **Extremely stressed**

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10

5. What was your level of happiness yesterday? Other ways to describe happiness include: joyful, glad, pleased.

Not happy **Extremely happy**

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10

6. How many times did you have a bowel movement or stool yesterday?

_______0 _______1 _______2 _______3 _______4
_______5 _______>5

7. How many different kinds of study foods did you eat yesterday?

_______0	_______1	_______2	_______3	_______4
_______5	_______6	_______7	_______8	_______9

Date:____________________________ **Subject Number:_________**

Final Visit Data Collection Sheet

(Study coordinators to complete this form with the help of the child and guardian)

Height:______________cm **BMI:**____________________________________kg/M^2

Weight:______________kg **BMI percentile:**________________________

Did you receive an influenza vaccination this school year (i.e., either in the fall or this spring). It could have been a shot (flu shot) or something they sprayed in your nose (Flu-Mist Nasal Spray).

No Yes – about when?__________________________________

Did you receive the swine (H1N1) flu vaccination this school year (i.e., either in the fall or this spring). This would be a shot and 2 shots are recommended.

No Yes – about when?__________________________________

Did you have 1 or 2 swine flu shots?_______________________________

Did you go to the doctor or clinic at any time during the study because you were not feeling well?

No Yes – What was not feeling well?____

Did you start taking any medications while you were helping us with the study?

No Yes – what were they?____

Did you miss any school days while you were helping us with the study?

No Yes – why did you miss?____

Which study group do you think you were in? Whole grain

Refined grain

Why do you think that?____

Subject Number:__________________________

Other Information

Weekly weights

post-study week 1 weight:______________kg Date:______________________

post-study week 2 weight:______________kg Date:______________________

post-study week 3 weight:______________kg Date:______________________

post-study week 4 weight:______________kg Date:______________________

post-study week 5 weight:______________kg Date:______________________

School recorded absences

Study participation dates:__________________________________

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:_________________Reason:____

Date absent:__________________Reason:____

Activities Scale

This purpose of this questionnaire is to estimate the amount of physical activity that you perform.

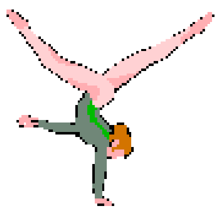
1. For each time period, write in the activity number that corresponds to the main activity you actually performed during that particular time period.

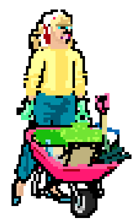
2. Then rate how physically hard each activity was. Place a "✓" in the timetable to indicate one of the following intensity levels for each activity.

• Light - Slow breathing, little or no movement.



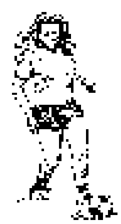

•Moderate - Normal breathing and some movement.



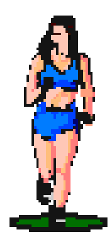
• Hard - Increased breathing and moderate movement.






• Very Hard - Hard breathing and quick movement.






Activity Numbers

Eating
1.) Eating a meal
2.) Snacking

Work
3.) Working (e.g., part-time job, child care) (list)______________________
4.) Doing house chores (e.g., vacuuming, dusting, washing dishes, animal care, etc.)
5.) Yard Work (e.g., mowing, raking)

After School/Spare Time/ Hobbies
6.) Church
7.) Hanging around
8.) Homework
9.) Listening to music
10.) Marching band/flag line/drill team
11.) Music lesson/playing instrument
12.) Playing video games/surfing Internet
13.) Reading
14.) Shopping
15.) Talking on phone
16.) Watching TV or movie

Transportation
17.) Riding in a car/bus
18.) Travel by walking
19.) Travel by bicycling

Sleep/Bathing
20.) Getting dressed
21.) Getting ready (hair, make-up, etc.)
22.) Showering/bathing
23.) Sleeping

School
24.) Club, student activity
25.) Lunch/free time/study hall
26.) P. E. Class
27.) ROTC
28.) Sitting in class

Physical Activities and Sports
29.) Aerobics/aerobic dancing
30.) Basketball
31.) Bicycling
32.) Bowling
33.) Calisthenics(i.e., jumping jacks, sit-ups)
34.) Cheerleading
35.) Dancing (social, recreational)
36.) Dancing (ballet, jazz, modern, tap)
37.) Field hockey
38.) Frisbee
39.) Golf
40.) Horseback riding
41.) Ice/roller skating
42.) Jogging/running
43.) Karate/judo/martial arts/ self-defense
44.) Rollerblading
45.) Skateboarding
46.) Soccer
47.) Softball/baseball
48.) Stationary exercise machines (e.g., cycle, ski machine, stair climber, treadmill)
49.) Street hockey
50.) Swimming, water exercise
51.) Tennis
52.) Volleyball
53.) Walking (briskly)
54.) Weight/circuit training
55.) Gymnastics/tumbling
56.) Kickboxing/Tae Bo
57.) Track and field
58.) Trampoline
59.) Other______________________

Sample activity time sheet:

The table below shows the correct way to fill out the activity time sheets.
Note that only **one** intensity level is checked for each activity.

	Activity Number	Light	Moderate	Hard	Very Hard
7:00-7:30	22	✓			
7:30-8:00	21	✓			
8:00-8:30	18		✓		
8:30-9:00	28	✓			
9:00-9:30	28	✓			
9:30-10:00	26			✓	
10:00-10:30	26			✓	

Put a "✓" to rate the intensity of each activity.

Write activity numbers in this column.

		Activity Number	Light	Moderate	Hard	Very Hard
before school	7:00-7:30					
	7:30-8:00					
during school	8:00-8:30					
	8:30-9:00					
	9:00-9:30					
	9:30-10:00					
	10:00-10:30					
	10:30-11:00					
	11:00-11:30					
lunch time	11:30-12:00					
	12:00-12:30					
	12:30-1:00					
	1:00-1:30					
	1:30-2:00					
	2:00-2:30					
	2:30-3:00					
	3:00-3:30					
after school	3:30-4:00					
	4:00-4:30					
	4:30-5:00					
	5:00-5:30					
supper time	5:30-6:00					
	6:00-6:30					
	6:30-7:00					
	7:00-7:30					
	7:30-8:00					
	8:00-8:30					
	8:30-9:00					
	9:00-9:30					
	9:30-10:00					
	10:00-10:30					
evening	10:30-11:00					
	11:00-11:30					
	11:30-12:00					

Targeted 24-hr Diet Recall

Subject number:_________________________________ Study coordinator: _________________________

Date these foods were consumed: ___________Day: S M T W Th F Sa **Page _____of ___**

Location (home/school/out)	Time	Meal	Food and Beverages Give Specific Details Get details on grains, fruit, &vegetables	AMOUNT and/or DIMENSIONS	Fresh frozen canned	Whole or refined grain? Brand?	COMMENTS

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BIOGRAPHICAL SKETCH

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