Impact of Whole Grains on Fecal Secretory IgA in Middle School students

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Abstract

Whole grains have a multitude of potential health benefits due to their high fiber, vitamin, mineral, and phytochemical content. Most adolescents do not consume the recommended servings of grains each day and few meet the recommendations for whole grain foods. This study investigated the effect of whole grains on fecal sIgA levels in adolescents consuming the recommended levels of whole- versus refined-grain foods. The relationship between fecal secretory immunoglobulin A (sIgA), the primary antibody found in mucosal secretions, and different weight categories (healthy weight vs. obese) was also investigated. Local middle school students participated in the study and were randomized to either a whole-grain or refined-grain diet. Participants gave a stool sample the week before the study began and also during week 6, the final week of the study. Whole grain intake at baseline was similar between both groups and increased significantly in those students receiving the whole-grain foods. There was no significant impact of whole grains on fecal sIgA levels; however, sIgA was higher in the feces from students in the healthy weight category compared to the obese weight category (Adjusted P = 0.0618). Future studies need to address the impact of lower concentrations of sIgA on health outcomes in obese adolescents.
Introduction

Background/Review of Literature

Whole grains, when eaten regularly, have the ability to confer numerous health benefits [1]. However, very few adolescents and adults eat at least three servings of whole grains per day, the amount recommended by the 2010 Dietary Guidelines for Americans [2]. Most adolescents do not even consume one serving of whole grain foods every day [2]. Whole grains, such as brown rice, wheat, and oats are a type of complex carbohydrate [3]. A whole grain is composed of an endosperm, bran, and germ. The endosperm of the grain is food for a potential growing seedling, which contains mostly starch and protein and forms the center of the grain. The germ and bran represent the regions surrounding the central endosperm and contain most of the compounds in whole grains that confer biological health benefits [3]. During a refining process called milling the germ and the bran of the whole grain are removed, leaving only the endosperm [4]. With this process most of the fiber, vitamin and mineral content, and phytochemicals are removed from the grain and with them the health benefits that whole grains may offer [3].

Those who frequently consume whole grain sources over refined grains may see decreased cardiovascular disease risk, improved insulin sensitivity, improved gastrointestinal health, reduction in hypertension, and many other health benefits [1]. The fibers, vitamins, minerals, and phytochemicals in whole
grains all have certain properties resulting in health benefits to those who eat them regularly.

**Benefits of fibers in whole grains**

The fiber content of whole grains can confer several major health benefits to individuals who eat a diet high in whole grains. By consuming a diet high in whole grains and fiber, microbial populations in the colon are altered and populations of beneficial bacteria outnumber harmful/neutral bacteria [5]. The bacterial composition of the colon can change with a high fiber diet and result in health benefits to the host [6]. When microbes are exposed to a high fiber environment, a large amount of fermentation occurs. Fermentation is a process where fibers are broken down and use for energy by certain bacterial populations [5]. Altering the microbial populations with a high fiber diet allows different byproducts to be produced. One such byproduct is the production of short chain fatty acids [6]. Butyrate, a specific short chain fatty acid, is thought to be a main reason why dietary fiber improves intestinal mucosal health. In vitro data suggests that butyrate may contribute to the ability of dietary fiber to reduce the growth and incidence of colorectal cancer by possibly altering DNA transcription [6]. High fiber diets have been shown to reduce overall gut inflammation due to byproduct production of microbial populations that ferment the fibers from whole grains for energy [5].

The physical presence of the fibers from whole grains in the colon causes a phenomenon called fecal bulking [7]. This bulking causes a dilution effect and may result in decreased risk for colorectal cancer due to diluting out any
carcinogenic chemicals that may be in the intestinal tract [7]. Colorectal cancer is the third highest cancer diagnosis behind lung and breast cancer [8]. A diet high in dietary fiber and whole grains has been associated with a reduced risk of being diagnosed with colorectal cancer [4, 6]. Along with fecal bulking, a diet high in whole grains and fiber has been observed to reduce the time food takes to pass through the body and be excreted, also known as transit time [7]. By increasing the rate that ingested carcinogens pass through the body they may be less likely to elicit their harmful effects on the mucosa [7].

Cholesterol reduction is seen from diets that are high in whole grain sources, which provide soluble fiber [1]. The soluble fiber binds to bile that is secreted by the gall bladder. This binding of the bile prevents it from being reabsorbed and used again by the body in a process called enterohepatic circulation. More bile has to be made by the body because some was excreted out of the body bound to fibers. Cholesterol is metabolized in the body for bile synthesis, and thus circulating cholesterol levels decrease [1]. Consumption of whole grains and high fiber diets has been associated with a decreased risk for being diagnosed with cardiovascular disease, though the exact mechanism by which this occurs is not yet clear [9].

Whole grain intake is inversely associated with Body Mass Index (BMI = weight [kg]/ height$^2$ [m]) [1]. Whole grains have the ability to help control body weight by causing feelings of satiety and thus reduction in overall food intake. Health conscious individuals may also be more likely to eat a diet high in whole grains. Obesity is increasing around the world both in children and adults [10].
Children who are obese have a high risk of developing metabolic disorder, type 2 diabetes mellitus, gastrointestinal diseases, sleep apnea, and other life altering conditions [10]. Compared to adults, children diagnosed with type 2 diabetes mellitus lose glycemic control at an accelerated rate [10]. Obese children who are diagnosed with type 2 diabetes mellitus have a decreased exercise capacity compared to obese children who do not have type 2 diabetes mellitus, further complicating weight loss goals [10]. Weight reduction has been shown to help control type 2 diabetes mellitus, sleep apnea, and other diseases associated with obesity [10].

**Benefits of vitamin and mineral content in whole grains**

Hypertension may be improved by consumption of whole grains because of the potassium content and low sodium content of whole grains compared to other foods [3]. When whole grains are added to a varied diet they may replace foods high in sodium and low in potassium. An inverse association has been discovered for the consumption of whole grains and frequency of incident hypertension in men [11]. The bran in whole grains seems to be the component that results in the hypertension reducing benefits associated with whole grains [11].

Whole grains are a source of vitamin E. Two forms of vitamin E exist in whole grains and nature [1]. These forms, tocopherols and tocotrienols, each have a free hydroxyl group that is the active site of the vitamin [1]. Vitamin E is a fat-soluble intracellular antioxidant, protecting polyunsaturated fatty acids from
being oxidized in the cell membrane [3]. Vitamin E also has the ability to prevent the formation of nitrosamines, most of which are carcinogenic compounds [3].

**Benefits of phytochemical content in whole grains**

Phytochemicals are a class of compounds that are not required by the human body for survival, but still elicit a useful function [3]. Whole grains contain many different types of phytochemicals, all of which have unique properties depending on their individual characteristics. The major types of phytochemicals in whole grains are carotenoids and phenolic acids [1].

Carotenoids, such as zeaxanthin and lutein, are found to be present in whole grains [1]. These phytochemicals are being implicated with health benefits ranging from reduced risk of being diagnosed with cardiovascular disease to cancer risk reduction due to their antioxidant properties [1].

Whole grains are also rich in a type of compound called phenolic acids, which have antioxidant properties [3]. Phenolic acids have the ability to act as free radical scavengers. Microorganisms in the colon ferment the fibers ingested in whole grains and release these compounds. The phenolic acids are then absorbed by the epithelial cells and antioxidant protection is conferred to the cell [3]. Ferulic acid is the most common phenolic acid in whole grains [1]. A conjugated double bond of the aromatic ring of ferulic acid has been said to be responsible for the antioxidant activity observed [1].

Phytochemicals are starting to emerge as a component of whole grains that may be adding to the reduced risk for cardiovascular disease being observed due to their antioxidant properties [1]. In the United States
cardiovascular disease is the leading cause of death and illness [12]. There was a 20-40% reduction in developing cardiovascular disease comparing groups of individuals who consumed whole grains regularly vs. those who did not when averaging the results from 17 studies on cardiovascular disease and whole grains [9].

**Fecal Secretory IgA as a biological marker of immune health**

The human immune system is extremely complex and dynamic. A strong immune system will lead to a decreased number of sick days and a healthier life, as the body will be able to combat infections more efficiently. At the heart of the immune system are immunoglobulins. These proteins are responsible for binding to antigens that may enter the body and eliciting an immune response. By finding a way to increase total concentrations of helpful immunoglobulins, such as secretory IgA (sIgA), human health may be improved [13].

The largest organ of the human body is the gastrointestinal (GI) tract. The large surface area of the GI tract is perfect for absorption of essential nutrients, however it is also a perfect location for invasion by foreign bacteria and viruses. As infectious agents pass through the intestine and colon they are met by immunoglobulins such as sIgA. Secretory IgA is the primary immunoglobulin that is excreted from mucosal cells, where it binds to antigens [14]. It is estimated that the human body produces approximately 66 mg of IgA per kg of bodyweight per day [15]. For a 100 kg male this would correspond to an average of 6.6 grams of IgA produced per day.
Secretory IgA is such an important immunoglobulin for the human immune system that it is secreted in large quantities in breast milk in order to protect the infant from infection while his/her own immune system is developing [16, 17]. Infants fed a prebiotic formula (i.e. a formula containing fermentable fibers) saw a trend of increased fecal slgA levels compared to infants fed a standard formula diet [18, 19]. This implies that supplementing with a high fiber diet, such as one found from consuming adequate quantities of whole grains, may improve fecal slgA levels. This topic will be investigated in this study with middle school students.

**Objective Aims**

As humans move through different stages of life, the immune system adapts and changes based on environmental conditions. A whole grain diet has been shown to decrease risk for certain diseases in adult populations [20]. Though this has been shown and supported in adults, studies involving children have been lacking. Childhood is a stage of life at which the body is very susceptible to infection. Childhood obesity has complicated the issue, as overweight individuals are at even higher risk for infection and metabolic disorders [10]. Middle school students participating in this study were divided into two groups. One group consumed a diet primarily of whole grain foods while the other ate a diet primarily consisting of refined grains. This study proposes that a diet high in whole grains will improve immune parameters, such as increased slgA secretion, in children from the grades of 6 to 8. Fecal slgA will be
used as a biological marker for immune health. The fecal sIgA levels of obese participants and healthy weight participants will also be compared. This study proposes that healthy weight participants will have higher fecal sIgA levels compared to obese participants.
Materials and methods

The University of Florida Institutional Review Board approved this study and all its advertisements. This study was a part of a larger study where fecal, blood, and saliva samples were taken from middle school students, along with daily questionnaires, in order to determine the effects whole grains have on adolescents. Inclusion criteria included parental/guardian consent, student assent, willingness of the student to eat three different study foods each day for 6 weeks, and willingness to provide 2 blood samples, 2 saliva samples, and 2 stool samples. Exclusion criteria included taking medications for constipation or diarrhea, taking antibiotics within 4 weeks of randomization, taking probiotics or consuming greater than 3 servings of yogurt per week, having food allergies, and having any disease/illness such as gastrointestinal disease, other chronic diseases, or immune-modulating diseases. Participants filled out informed consent forms with permission from parents in order to participate in the study. The middle school students who were approved for the study and filled out the informed consents were randomized and separated into either a refined grain or whole grain diet. Each student was separated into one of three weight categories based on their BMI percentiles. A BMI of less than 85th percentile classified that student as healthy weight. A BMI between 85th and 95th percentile classified that student as overweight. A BMI of greater than 95th percentile classified that student as obese. Study foods were provided for the participant weekly for daily consumption. Participants were required to eat at least 3 servings of their study foods every day for six weeks.
Storage

Students gave fecal samples before the study commenced and during week 6. Fecal samples were collected within 4 hours of defecation. Samples were homogenized and subsequently stored in 50 ml conical tubes at -70 °C.

Preparation/extraction

One milliliter of ELISA wash buffer was added to 15 ml tubes and weighed by placing in the tube in a 50 ml beaker. The weight of the tube and buffer combined was recorded for the initial measurement. Fecal samples stored at -70 °C were allowed to thaw for 5 minutes before samples were used. Between 80 – 120 mg of sample was extracted and added to the 15 ml tube. The weight was taken again after the sample was added and subtracted from the initial weight to determine the weight of the extracted sample. The weight of the sample was recorded and an additional amount of wash buffer was added to each tube using the equation [Buffer added (ml) = (Sample weight/20) – 1] to bring the stool mass to a final concentration of 20 mg/ml.

After the sample was diluted into the proper wash buffer volume the 15 ml tubes were mixed thoroughly for 30 seconds using a Fisher Vortex Genie 2. One milliliter of the stool suspension was transferred to an Eppendorf-tube and centrifuge for 5 minutes at 13000 rpm (~ 1300 g). Supernatant was not stable and thus could not be stored for future use.
Stool Dilution

After centrifugation the supernatant was diluted 1:250 in wash buffer. A 100 μL aliquot of the diluted solutions were used for analysis with Enzyme Linked Immunosorbent Assay (ELISA).

Plating

The fecal samples were analyzed using ELISA. The following steps were used to plate all of the diluted fecal samples for analysis: the pre-coated plate was washed 5 times with 250 μL ELISA wash buffer. One hundred μL of standards, control, and samples were added to wells. The plate was incubated for 1 hour at room temperature, while shaking on a horizontal mixer. The contents of the plate were aspirated and the wells washed 5 times with 250 μL ELISA wash buffer. One hundred μL of conjugate (mouse anti-sIgA, Peroxidase-labeled) was added. The plate was incubated for 1 hour at room temperature, while shaking on a horizontal mixer. The contents of the plate were decanted and the wells were washed 5 times with 250 μL ELISA wash buffer. One hundred μL of TMB substrate was added to each well. The plate was incubated for 10 – 20 minutes at room temperature. Fifty μL of STOP (ELISA stop solution) was added and mixed shortly before plate reading.

Plate reading

Absorption was determined with an ELISA reader, immediately after the short mixing step was complete, at 450 nm against 620 nm as reference. If the
extinction of the highest standard exceeded the measurement range of the photometer, absorption was measured immediately at 405 nm against 620 nm as reference. Results were saved and backed up on a separate hard drive for further analysis. Pre-baseline and final week fecal samples were analyzed for sIgA using ELISA. Data were analyzed using mixed effects linear models with time period, treatment group, weight category (from baseline weight), and their interactions as fixed effects and subject as a random effect. Differences between means were tested using the Tukey-Kramer adjustment.
**Results**

Eighty three participants were randomized to either a whole grain \((n = 41)\) or refined grain \((n = 42)\) diet (Table 1). No adverse effects were reported and all students finished the study. Whole-grain intake was similar between groups during the pre-baseline period and significantly increased in the whole-grain group and decreased in the refined-grain group (Table 1). Across groups, total grain intake increased by 1 oz equivalent during the intervention (data not shown). Not all subjects provided a stool sample. Fifty eight baseline and sixty eight final stool samples were obtained.

No significant interaction between treatment group and change in mean slgA levels pre-baseline to final was observed (Figure 1A). An effect with fecal slgA was only seen with baseline weight category \((P=0.0448)\) after being analyzed as a function of treatment group, period, and baseline weight category. Fecal slgA levels were higher across treatment groups in healthy weight participants compared to obese participants (Multiple Comparisons: Tukey-Kramer, Adjusted \(P = 0.0618\)) (Figure 1B).
Table 1. Demographics of middle school students randomized to a whole or refined grain diet.

<table>
<thead>
<tr>
<th></th>
<th>Refined-Grain Group (n=42)</th>
<th>Whole-Grain Group (n=41)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>25/17</td>
<td>23/18</td>
<td>NS¹</td>
</tr>
<tr>
<td>Age in years</td>
<td>12.7±0.2²</td>
<td>12.6±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Grade in school</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6th (n[%])</td>
<td>19 (45%)</td>
<td>22 (54%)</td>
<td>NS</td>
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<tr>
<td>7th (n[%])</td>
<td>15 (36%)</td>
<td>9 (22%)</td>
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</tr>
<tr>
<td>8th (n[%])</td>
<td>8 (19%)</td>
<td>10 (24%)</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity (n[%])³</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td>18 (43%)</td>
<td>20 (49%)</td>
<td>NS</td>
</tr>
<tr>
<td>Black/African American</td>
<td>14 (33%)</td>
<td>12 (29%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>6 (14%)</td>
<td>5 (12%)</td>
<td></td>
</tr>
<tr>
<td>Mixed/Other</td>
<td>4 (10%)</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td>BMI percentiles (n[%])</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>&lt; 85th</td>
<td>23 (55%)</td>
<td>23 (56%)</td>
<td></td>
</tr>
<tr>
<td>85th – 94th</td>
<td>10 (24%)</td>
<td>6 (15%)</td>
<td></td>
</tr>
<tr>
<td>≥ 95th</td>
<td>9 (21%)</td>
<td>12 (29%)</td>
<td></td>
</tr>
<tr>
<td>Whole grain intake (g)⁴</td>
<td></td>
<td></td>
<td>&lt;0.0001⁵</td>
</tr>
<tr>
<td>Pre-baseline</td>
<td>18.4±4.2</td>
<td>17.7±3.4</td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>3.6±0.7</td>
<td>60.4±5.0</td>
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<tr>
<td>Fiber intake (g)⁴</td>
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<td>0.0083⁵</td>
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<tr>
<td>Pre-baseline</td>
<td>12.1±1.2</td>
<td>10.7±0.9</td>
<td></td>
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<tr>
<td>Intervention</td>
<td>10.1±0.5</td>
<td>13.1±0.9</td>
<td></td>
</tr>
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</table>

¹Not significant, NS.
²Data represent the mean±SEM. Differences between groups were analyzed using a t test.
³The “other” category consisted of Asians (n=2), mixed White/African American (n=4) and other (n=2). Participants who self-defined themselves as White and American Indian/Alaskan Native (n=2) or White and Native Hawaiian/Pacific Islander (n=1) were classified as White. The participant who self-classified as Hispanic and American Indian/Alaskan Native was classified as Hispanic. Categorical data were compared by using the chi-square statistic.
⁴Intakes represent dietary intake from all foods (i.e., study and non-study foods). Data represent means±SEM.
⁵P value for the interaction between group and time (i.e., pre-baseline vs. intervention).
Figure 1: Mean fecal sIgA concentrations by treatment group (A) and pre-baseline weight category. No interaction between fecal sIgA levels and treatment group was seen (Pr > F = 0.5004) (A). Fecal sIgA levels by participant weight category were compared (B). Differences between means were tested using the Tukey-Kramer adjustment found a significant difference in fecal sIgA levels between healthy weight and obese weight categories (Adjusted P = 0.0618) (B).
Discussion

In examining the effects of whole grains on fecal sIgA levels there were no significant changes with fecal sIgA between the refined group and the whole grain group. This was not the expected outcome, however it may be explained by the bulking effect that whole grains have in the colon.

A whole grain diet has been known to cause “fecal bulking” in the colon by bringing in water and causing a diluting effect. This diluting effect may have skewed the fecal sIgA measurements because the samples were initially diluted by total weight and not by dry mass (in which the water was removed from the sample before weighing). A whole grain fecal sample may have less “dry” mass and more “wet” mass when compared to the same volume of a refined grain fecal sample. This dilution effect may have skewed the data and caused no effect to be observed when there may have been one if dry mass was used.

When analyzing baseline weight category there was a significant difference between fecal sIgA levels between healthy weight and obese individuals. The obese group had the lowest fecal sIgA levels. This was expected and may suggest a difference in metabolic function and overall health at the different weight categories. Children who are obese have a high risk of developing metabolic disorders and other life altering conditions [10]. Obesity has also been associated with decreased immune function, thus lower sIgA levels would be expected compared to healthy weight individuals [21].

Fecal sIgA and other immunoglobulins may be used as markers for immune health due to their importance in the defense of our body from foreign
pathogens. A diet high in whole grains may have an effect on fecal sIgA levels if
the bulking effect of the whole grains is taken into account. Finding a way to
improve immune parameters may lead to a healthier life, less missed days from
school or work, and less economic loss to businesses that rely on healthy
workers. The implications for immune boosting dietary changes are vast and
should be investigated further for possible reduction in illness.
References


10. Abrams P, Levitt Katz LE: **Metabolic effects of obesity causing disease in childhood.** *Curr Opin Endocrinol Diabetes Obes* 2011, **18**:23-27.


