Effects of Dietary Fibers on Obesity Related Physiological Parameters of Mice

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ABSTRACT

Obesity, a metabolic disease resulting from an imbalance between caloric intake and expenditure, is a global concern. This study investigated the effect of dietary fibers (glucomannan, GM and oat β-glucan, OBG) on obesity related variables, like body and liver growth, serum metabolites, fatty liver (steatosis), and short chain fatty acid production in eighty-four high-fat diet-induced obese male, C57BL/6 mice. At 6-weeks old, the mice were fed one of 7 diets (n=12): Control, 1.25%, 2.5%, 5% GM or OBG for 12 weeks. The GM diets reduced body weight gain ($P<0.005$) with an interaction between fibers and their inclusion levels for relative liver weight ($P<0.05$) and percent steatosis ($P<0.001$). The OBG diets had lower serum triglyceride concentrations ($P<0.05$), while the GM diets had higher acetate and propionate production ($P<0.05$) compared to the other dietary treatments. At the 1.25% inclusion level, microvesicular fat was prevalent in both GM and OBG diets compared to the other dietary treatments at 2.5% and 5%. In conclusion, glucomannan and oat β-glucan at specific inclusion levels exert significant effects on relative liver weight, percent steatosis, and serum triglycerides in the mice. Glucomannan decreased the severity of mediovesicular fat, while both fibers decreased severity of macrovesicular fat. Thus, supplementing a diet with an adequate amount of specific dietary fiber may improve obesity related health issues.
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CHAPTER 1: INTRODUCTION

1.1 Current descriptive and analytical definitions of dietary fiber

The definition of dietary fiber (DF) has evolved tremendously over the past 50 years with ongoing research investigations and nutrition-related discoveries in the health field. Though, the universal definition of DF has become quite complex with research showing DF benefits coming not just solely from a plant’s cell wall components. A wide range of chemical, physical, and physiological properties must be acknowledged in defining DF, which has naturally led to descriptive and analytical challenges.

Previous definitions did not capture all the non-digestible material in food (Jones, 2013). In short, Hipsley (1953) coined the term “dietary fiber” to describe the non-digestible plant cell wall constituents that included lignin, cellulose, and hemicelluloses. Then by the early 1970s, a team of British physicians recognized a higher prevalence of certain diseases (diverticular disease, colon cancer, appendicitis, and heart disease) in Western countries compared to African countries to suggest that these diseases could have been prevented or reduced with a daily intake of DF. This led to the emergence of the DF hypothesis, where Trowell (1972) defined DF “as the skeletal remains of plant cells that are resistant to hydrolysis by the enzymes of man.” This definition was too specific because it only showed a physiological aspect of DF being indigestible in the human gastrointestinal tract (DeVries et al., 1999). To suit a more chemical aspect, the dietary definition broadened to “all polysaccharides and lignin that are resistant to hydrolysis by animal digestive enzymes” (Trowell et al., 1976).

The Codex Alimentarius Commission is a joint cooperation between Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) that agrees on food standards, guidelines, and codes of practice to meet the safety, quality, and fairness in international food trade (Moller, 2011). In 2009, the Codex DF definition was finally accepted after 16 years of deliberations. It has been defined as: carbohydrate polymers with 10 or more monomeric units that are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: 1) edible carbohydrate polymers naturally occurring in the food as consumed; 2) carbohydrate polymers that have been obtained from food raw material by physical, enzymatic, or chemical means and have been shown to have
a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; and 3) synthetic carbohydrate polymers that have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities (Jones, 2013). However, it depends on the country to keep the degree of polymerization (DP) at a limit of 10 monomer units. European countries have decided to include DP from 3-9 (Moller, 2011). Currently in the United States, the descriptive definition for DF states that it consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. While functional fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Together, DF and functional fiber make up total dietary fiber (TDF) (McCleary et al., 2010). These updated definitions did not just include one single DF entity. With varying physical and physiological effects on DF, the definition had to broaden, as well as the addition of analytical methods to characterize them all.

Dietary fiber in the United States has been defined by several analytical methods that are allowed by Association of Official Analytical Chemists (AOAC) (Institute of Medicine, 2005). The development of analytical methods helped quantify DF in food products over the years to improve nutritional needs and labeling purposes. In 1981, a general consensus was achieved at the AOAC workshop in Ottawa, Canada, where collaborative studies with the support of many laboratories across the globe, established the first AOAC Official Method 985.29: Total Dietary Fiber in Foods-Enzymatic-Gravimetric method (Devries et al., 1999). Modified from method 985.29, AOAC Official Method 991.43 is currently the accepted method of analysis in the United States that determines not only TDF, but also soluble and insoluble DF in food products (McCleary et al., 2010). Moreover, the AOAC 2009.01 determines the measurement of TDF in addition to resistant starch (Moller, 2011).

1.2 Dietary fiber classifications

Dietary fiber definitions based on analytical methods are generally characterized by the chemical composition of DF. However, the inability to predict biological and physiological responses of DF once it is consumed is a limitation. This leads to TDF being the sum of DF and functional fiber (Dikeman & Fahey, 2007). Dietary fibers are classified based on their source (plant, animal, isolated, synthetic), chemical makeup, physical properties, and physiological
effects (Jones, 2013). Gathering information from each component serves a purpose that can potentially explain the interaction between DF and biological functions.

### 1.2.1 Physico-chemical

Dietary fibers have been chemically classified based on their chain length, DP, and the type of linkages between each monomer unit (α or β). The designated chain length was a DP ≥ 10 (ex: polysaccharides), however, it was up to the national authorities to decide if they wanted to include carbohydrate polymers with a DP < 10 (like oligosaccharides or short chain carbohydrates) (Jones, 2013). Authorities have recommended a DP of 10 as the dividing point of establishing carbohydrates as being oligo- or polysaccharides (IUB-IUPAC and Joint Commission on Biochemical Nomenclature, 1982). This DP issue put the international DF consensus at a disadvantage because there were many discrepancies in research findings, nutrition labels, and food composition tables suggesting discoveries based on DF with varying chain length. In general, polysaccharides can be divided into resistant starch (α-1,4 and 1,6 glucan linkages) and non-starch polysaccharides (NSP), which are mainly cellulose, hemicelluloses, and pectin. Non-starch polysaccharides are from plant cell walls that are usually formed by β-linkages (Cummings and Stephen, 2007; Jones, 2013).

One property of DF is its solubility. Soluble DF attract water and forms a gel to slow down digestion, which delays the emptying of stomach and creates a fullness effect. This interferes with macronutrient absorption, reduces postprandial glucose responses, and reduces total cholesterol levels (Isken et al., 2010). Another main property of DF is its viscosity, which is its resistance to flow that allows fibers to thicken when mixed with fluids. Some examples of some viscous DFs include soluble polysaccharides such as gums, pectins, psyllium, and β-glucans. In addition, the Food and Drug Administration (FDA) of United States accepts that health benefits of soluble fiber are associated with its viscosity. The viscosity level requirement for each type of DF to achieve these glucose-lowering effects is poorly understood. Previous studies have compared viscosity levels between different fibers, but only a few have been compared within the same type of fiber (Panahi et al., 2007). By altering the viscosity of digesta in the gastrointestinal tract, the absorption of nutrients such as glucose and cholesterol are inhibited (Dikeman & Fahey, 2006). Viscous DFs delay gastric emptying as it hinders food bulk
diffusion to the small intestine. Viscous fibers have been proven to lower postprandial glucose and cholesterol levels (Dikeman & Fahey, 2006; Brockman et al., 2014).

Another property of DF is its fermentability associated with large intestine functionality. Non-digestible food components like peptides, protein, oligosaccharides, polysaccharides, and glycoprotein precursors are resistant to hydrolysis, as well as digestion in the stomach and small intestine until they reach the large intestine. Highly fermented DFs are broken down into metabolizable energy in the form of short chain fatty acids (SCFA) to help regulate intracellular pH levels, ion transportation, and cell differentiation of colonic epithelium, in addition to improving systemic health. This anaerobic process is called microbial fermentation (Wong et al., 2006). Studies have shown that acetate, propionate, and butyrate are the three most common organic SCFAs, making up approximately 80% of SCFAs with 90% of these being rapidly absorbed efficiently in the cecum and colon at different rates with only 5-10% being excreted in human feces. The quantity of SCFA produced through fermentation is dependent on the species observed, the bacterial population count present in the colon, the source of DF, and the gut transit time (Wong et al., 2006). The majority of the SCFAs remaining are transported to organs for specific biological regulatory mechanisms.

Acetate, propionate, and butyrate are the three primary SCFAs that are produced by gut microbiota from complex dietary carbohydrates. Once available in the gut lumen, these SCFAs are absorbed by colonocytes for various functions. Acetate is the principal SCFA in the colon that is quickly transported to the liver for lipogenesis that function as a substrate for acetyl-CoA formation in the cytosol that increases lipid and cholesterol synthesis. Propionate is a substrate for liver gluconeogenesis that inhibits cholesterol synthesis in the liver. However, acetate and propionate have shown inconsistent effects. Butyrate provides some energy to help maintain cell proliferation and differentiation for colonic epithelial cells. These three metabolites not only provide energy, but also are considered signaling molecules (Wong et al., 2006; Immerstrand et al., 2010; Lin et al., 2012).

1.2.2 Physiological

Dietary fibers that are resistant throughout the digestion process are plant cell wall polysaccharides, gums, fructans, resistant maltodextrins, and resistant starches. Amylases break down sugars from the food bolus, which determine blood glucose levels. Once they enter the
large intestine, gut bacteria ferment the sugars and produce SCFA (Khoury et al., 2012). Based on the physiological property alone, it does not provide an in-depth analysis on DF. Although, carrying out these established DF classifications of chemical, physical, and physiological properties help us get closer to comprehending research findings.

1.3 Prevalence of obesity

Obesity is a chronic disease increasing in both severity and prevalence in many modern-day societies. It is generally caused by an excessive caloric intake with low physical activity, resulting in less calories being eliminated from the body (Hill et al., 2012). An increasing trend in over-nutrition in the past several decades has been recognized in first world countries, mainly the United States. Major health problems stemming from obesity include increased risks in insulin resistance, diabetes, hypertension, metabolic syndrome, and coronary heart disease (Dandona et al., 2005). More specifically on metabolic syndrome, it is characterized by chronic inflammation, elevated triglyceride concentration, and hepatic steatosis or non-alcoholic fatty liver disease (NAFLD) (Bose et al., 2008). With this cluster of numerous risk factors coming from obesity, it is important to investigate and research biological mechanisms of obesity to develop useful and realistic approaches to prevent its occurrence.

According to the Centers for Disease Control and Prevention (CDC, 2012), overweight and obesity are both terms pertaining to weight ranging greater than a healthy person for a given height. Being overweight or obese has been proven to increase the chance of health complications and a reduced life expectancy. Both of these inhibit normal health functions in the human body with its characterized excessive body fat accumulation. As of 2014, WHO estimated that 2 billion adults, being 18 years and older were overweight with 600 million of them being obese. Moreover, 42 million children under the age of 5 were either overweight or obese in 2013 with an increase rate of 30% higher in developed countries than developing countries with emerging economies. On a global scale, the number of obese people has doubled between 1980 and 2014 (WHO, 2015).

A major risk factor of obesity is diabetes. There is Type I diabetes, an autoimmune disease that destroys insulin-producing pancreatic beta cells, which leads to hyperglycemia. Patients must take subcutaneous insulin injections frequently and monitor their blood glucose levels (King, 2012). This is usually diagnosed in children and young adults, making up 5% of the
diabetic population (American Diabetes Association, 2015). Meanwhile, Type II diabetes affects 95% of the diabetic population and is characterized by insulin resistance. Its occurrence directly increases with body mass index (BMI), with heredity playing a role in its development. However, the interaction between genetics and dietary intake has not been fully understood (King, 2012). In addition, the degree of obesity will differ among people based on genetics and environmental factors. Genetic contribution to obesity has ranged from 5 to 25% with environmental factors of low income and a low level of education being major factors in the development of obesity (Kimm, 1995).

Health care organizations and professionals have demonstrated strategies to prevent the beginning stages of obesity and diabetes by following an exercise routine and eating a balanced nutritional diet. However, those two attempts to prevent obesity have had a limited success rate (Yazdi et al., 2015). Methods in preventing obesity-related diseases are constantly being investigated because controversial positions exist among studies using animal models and human trials (Galisteo, et al., 2008). Sometimes it is easier for patients to pursue drug therapy. There are drugs that can stimulate insulin production, decrease liver glucose production, or slow the process of carbohydrate uptake in the gastrointestinal tract (King, 2012). However, the limitation to taking prescribed medicine is that it is not as simple as following one single nutrient, but focusing on the interaction between many nutrients and their effect on the entire biological system for both diet intervention and drug therapy.

The role of DF has shown to be a useful treatment for obesity, depending on its chemical composition and fiber type. Fiber supplemented into a balanced diet has been suggested to increase satiation that slows gastric emptying. This allows a person to feel a fullness effect without consuming a calorie-dense meal. More specifically, viscous DFs create a gel-like form that has been effective in reducing levels of low-density lipoprotein (LDL) cholesterol without altering the high-density lipoprotein (HDL) levels (Smith, 1987; Gunness & Gidley, 2010).

The average DF intake for United States children and adults are less than half of the recommended intake levels (Anderson et al., 2009). The recommended daily DF intake for adults age 50 or younger is 38 g for men and 25 g for women, while adults age 51 or older is 30 g for men and 21 g for women (Institute of Medicine, 2012). It has been recognized that individuals consuming a fiber-rich diet are at a significantly lower risk for developing cardiovascular disease, diabetes, hypertension, obesity, and gastrointestinal diseases. Studies have shown that
the increase in fiber intake help lower blood pressure, lowers serum cholesterol levels, and improves insulin sensitivity (Anderson et al., 2009). For now, consuming more DF in meals will help aid the health issues associated with obesity. Although the effect of DF components and how it interplays with obesity symptoms are still not completely understood. Nutritionists, health professionals, researchers, organizations, and educators need to influence communities with their findings to lessen the knowledge gap.

1.4 Dietary fiber and fatty liver disease

Non-alcohol fatty liver disease also known as hepatic steatosis is the most common liver disease in the absence of alcohol consumption in the United States. It is a major health burden strongly associated with the risk factors of type II diabetes mellitus, insulin resistance, and obesity. Therefore, early detection is crucial for the treatment of NAFLD symptoms that could potentially bring about those associating risk factors. It is necessary to start with lifestyle modifications through increased physical activity, seen similar in obese patients. Regulating NAFLD reduces body weight that usually helps improve serum liver enzymes, reduces liver fat infiltration, and more serious NAFLD complications including inflammation and fibrosis. However, maintaining a healthy body weight has shown to be more difficult than modifying dietary intakes. Research is still emerging because it still is unclear whether diets supplemented with certain foods or nutrients are more likely to develop hepatic steatosis compared to other types of diets (Zelber-Sagi, et al., 2011).

Non-alcohol fatty liver disease is characterized by having hepatic lesions with macrovacuolar steatosis, microvesicular steatosis, necroinflammation, and/or apoptosis (Tolman and Dalpiaz, 2007; Trak-Smayra et al., 2011). More specifically, steatosis is the accumulation of fat in the form of triglycerides within hepatocytes (liver cells). The presence of >5% of the hepatocytes with steatosis is the minimum qualification that is accepted for a histological diagnosis of NAFLD (Brunt and Tiniakos, 2010).

The NAFLD steatosis spectrum ranges from 1) pure fatty infiltration, 2) fatty infiltration with inflammation, 3) necrosis with or without fibrosis (steatohepatitis), and 4) cirrhosis (progress to liver failure) as a result of excessive alcohol consumption (Ludwig et al., 1980). The spectrum of vesicular fat residing in hepatocytes ranges in different sizes. Macrovesicular steatosis is distinguished by hepatocytes having a single, large fat vacuole within the cytoplasm
that displaces the nucleus more toward the outside of the hepatocyte (Brunt and Tiniakos, 2010). Microvesicular steatosis is defined by having tiny cytoplasmic lipid droplets around a centrally positioned nucleus, while several medium-sized lipid droplets present in the cytoplasm of the hepatocytes is called mediovesicular steatosis (Trak-Smayra et al., 2011). Steatosis evaluation has been divided into three observational categories depending on the severity level of the hepatocytes: 0-33% (mild), 33-66% (moderate), and 66-100% (severe) (Brunt and Tiniakos, 2010).

In general, early stage of NAFLD steatosis does not show many symptoms of liver disease. Liver biopsy has shown to be much more reliable compared to ultrasound, computerized tomography scan, and magnetic resonance imaging in identifying inflammation or fibrosis in the liver (Clark et al., 2002). Insulin resistance is one of the hepatic abnormalities that stimulate glucose production in the liver. The increase in glucose causes hepatic lipogenesis, causing altered biological functions during obesity. While hepatic issues persist, NAFLD also arises with many other liver damages ranging from relatively benign hepatic steatosis to potentially fatal cirrhosis (Clark et al., 2002; Brockman et al., 2014).

From the available data from epidemiological studies, identifying and modifying daily dietary intake of nutrients and foods can potentially improve the symptoms of NAFLD. Unbalanced nutrition is a major risk factor of NAFLD and its health complications. It is important to reduce saturated/trans fat and increase polyunsaturated fat, mainly omega-3 fatty acids. In addition, avoid carbonated beverages, reduce drinking high fructose containing juices, and increase fiber intake. However, most human studies have been only observational, limiting the understanding and not allowing for inferencing the causal relationships between nutrition and its association with NAFLD (Zelber-Sagi, et al., 2011).

1.5 Characteristics of glucomannan

Glucomannan is a highly-branched heteropolysaccharide composed of β-D-(1,4) linked glucose and mannose units, giving this DF its very high molecular weight (Gallaher et al., 2000; Takigami, 2000; Gonzalez et al., 2004;). Konjac glucomannan makes up about 8-10% of the raw konjac tuber (root) that also has starch, lipids, and minerals (Takigami, 2000).

Glucomannan is an off-white powder extracted from the dried root of the Amorphophallus konjac plant C. Koch, a member of the family of Araceae (Nishinari et al.,
For many centuries, nine *Amorphophallus* species of the 170 total species have been used for food, medicine, and even wine production in China, Japan, and other South East Asian countries. In ancient Chinese medicine, the konjac plant’s tuber was rinsed, peeled, sliced, dried, and then grounded into flour. After boiling the konjac flour, a gel-like cake formed for easy consumption and its extract demonstrated therapeutic effects like detoxification, tumor-suppression, blood stasis alleviation, and phlegm liquification. For thousands of years, it has been used to treat asthma, cough, hernias, breast pain, burns, blood and skin disorders (Chua et al., 2010).

In 6th century A.D., konjac was introduced to the general public as medicine in Japan with T. Nakajima (1745-1826) initially creating commercial purified konjac processing technologies by pulverizing dried chips of the konjac tuber. Then, K. Mashiko (1745-1854) further looked into this method to produce cleaner konjac flour, commonly known as konjac glucomannan (KGM) by polishing the konjac tuber with a mortar that separated the impurities from the konjac flour by wind sifting (Takigami, 2000). It was commonly used to stabilize traditional Japanese noodles in boiling water (Nishinari et al., 1992). Later in 1994, konjac flour was introduced and approved by the FDA to be a food-thickening agent for gravies, soups, and sauces in the United States and Europe. It has helped with texturizing and water binding in food recipes (Chua et al., 2010; Takigami, 2000). Over the years, KGM has proven to show great importance as a DF that enhances texture, water absorption, capacity, and viscosity. In the biopharmaceutical field, this DF has been considered to be advantageous in weight loss, decreasing in cholesterol levels, decreasing in carbohydrate absorption, increasing in stability, enhancing in protein association, and recognizing mannose receptors. There is a possibility of using KGM in the drug delivery field, but more preparation and discussion needs to take place before anything substantial comes into play (Alonso-Sande et al., 2008).

### 1.6 Characteristics of beta-glucan

Beta-glucans have been one of the most documented and investigated DFs in the past 30 years. These are linear polysaccharides with β-(1,4) and β-(1,3) linkages found typically in the cell walls of oats, barley, wheat, and rye (Immerstrand et al., 2010). Soluble fiber from the soluble fraction of α-amylase hydrolyzed oat bran or whole oat flour is referred to as oatrim. Oats are one of the first functional foods approved by the FDA with its health claim that states 1)
coronary heart disease continues to be a disease for which the United States population is at risk; 2) soluble fiber from oatrim when used at levels providing 0.75 grams of β-glucan soluble fiber per serving is a food because it provides nutritive value; 3) oatrim when used at levels necessary to justify the health claim is safe and lawful; 4) there is a physiological equivalence of β-glucan soluble fiber from oatrim and of whole oat sources such as oat bran and rolled oats; and 5) there is significant scientific agreement among qualified experts that oatrim with a β-glucan content of up to 10% on a dry weight basis and not less than that of the starting material may reduce the risk of coronary heart disease (FDA, 2003). Common food products with the addition of β-glucans are baking products, bread, pasta, milk products, salad dressings, and noodles that have shown effects of water binding, emulsion stabilization, thickening, and being a texturized substance. The content of β-glucans depend on endosperm cell wall degradation during germination (Khoury et al., 2012).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects (n)</th>
<th>Duration of diets</th>
<th>Dietary Treatments</th>
<th>Significant Findings</th>
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<tbody>
<tr>
<td>Gallaher et al., (2000)</td>
<td>Wister rats (32-36); male</td>
<td>18 days</td>
<td>- Modified AIN-93G diet w/ 0.125g/100g cholesterol and 7.5g/100g of test materials: 1) chitosan (CH), 2) glucomannan (GM), 3) equal mixture of CH + GM, and 4) cellulose (control)</td>
<td>- Total liver cholesterol reduced in GM, CH, and CH + GM groups compared to control. Daily fecal fat excretion was higher in CH + GM and CH compared to GM and control. Although, cholesterol lowering for both GM and CH seem to be from different mechanisms.</td>
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<tr>
<td>Bae et al., (2009)</td>
<td>C57BL/6 mice (50); 4 weeks old; male</td>
<td>6 wks</td>
<td>1) Normal: AIN-76A purified rodent diet w/ 65% corn starch 2) High fat diet (HFD): modified AIN-76A w/ 40% beef tallow and 3-5) HFD supplemented w/ β-glucans at 3 different molecular weights (MW) of 1450, 730, and 370 x 10^3 g/mol</td>
<td>- Experimental HFD w/ β-glucans reduced body weight (BW) of mice and helped improve the serum lipid profile (total lipid, triglyceride, and total cholesterol levels. Although, these effects did not seem to be caused from the different molecular weights.</td>
</tr>
<tr>
<td>Gao et al., (2009)</td>
<td>C57BL/6J mice; 4 weeks old; male</td>
<td>12 wks</td>
<td>1) HFD w/ 5% sodium butyrate (SB) 2) HFD (control)</td>
<td>- HFD w/ SB significantly lowered BW, body fat content in % BW, while increasing energy expenditure.</td>
</tr>
<tr>
<td>Study</td>
<td>Mouse Strain &amp; Gender</td>
<td>Diet Details</td>
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<td>Immerstrand et al., (2010)</td>
<td>C57BL/6NCrl mice; female 10-12 wks old (110)</td>
<td>4 wks 1) Oat bran diets with different peak MW of β-glucans (2348, 1311, 241, 56, 21 or &lt; 10 kDa) – all diets reflected the Western diet (41% energy fat) and designed to be atherogenic (0.8% cholesterol and 0.1% cholic acid)</td>
<td>- BW increased in mice fed oat products. - All OBG diets (regardless of MW) reduced plasma cholesterol compared to control (cellulose included) diet. No effect on cholesterol-lowering properties from the different viscosity proportion of OBG diets. No effect in plasma triacylglycerols (TAG). Significant proportional increase w/ ratio of (propionic acid + butyric acid)/acetic acid and the MW of β-glucans. - BW and SCFA production increased in mice fed soluble diet ($P=0.03$ and $P=0.002$, respectively). No effect in fasting plasma cholesterol, TAG, glucose, and insulin. - BW of both mice groups increased in both diets. Microvesicular fat was more frequently observed in db/db mice compared to ob/ob mice in whichever diet. HC diet increased severity of</td>
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<td>Isken et al., (2010)</td>
<td>C57BL/6J mice (14) 16 weeks old; male</td>
<td>45 wks 1) 10% (w/w) soluble guar gum 2) 10% (w/w) insoluble cereal fiber</td>
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<td>Trak-Smayra et al., (2011)</td>
<td>C57BL/6J-ob/ob mice (40)</td>
<td>1 month &amp; 3 months 1) Standard chow (16% proteins, 3% fat, 60% carbohydrates) (A04) 2) High-calorie (HC) diet (Western diet: 16% proteins, 16% fat, 46% carbohydrates)</td>
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<tr>
<td>Study</td>
<td>Genotype/ Age/ Sex</td>
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</table>
| Tian et al., (2013)   | C57BL/6 mice (40); male | 12 wks   | 1) HFD w/ 4% β-glucan (from highland barley  
2) HFD w/ 2% β-glucan  
3) HFD & 4) Standard diet (control)  
4) Low fat diet (LFD) w/ cellulose (3.9kcal/g) |
| Zhang et al., (2013)  | C57BL/6 mice (32); 6 weeks old; female | 6 wks    | 1) HFD w/ 2% Salecan (64% energy)  
2) HFD w/ 5% Salecan  
3) HFD w/ cellulose (control) (5.6kcal/g)  
4) Low fat diet (LFD) w/ cellulose |
| den Besten et al., (2014) | C57BL/6J mice; (32); 2 months old; male | 6 wks    | 1) - 4) HFD supplemented w/ 0%, 5%, 7.5, or 10% (w/w) of guar gum fiber |

- Steatosis in db/db mice after 1 month, but decreased after 3 months. HC diet increased total cholesterol (TC) in db/db mice. No effect in triglycerides, glucose, and antioxidant status.  
- The supplemented HFD with β-glucan reduced serum glucose and triglycerides, as well as improve insulin resistance.  
- HFD-2%S and HFD-5%S lowered BW by 11% and 18% compared to HFD, respectively. HFD-5%S decreased liver weights by 22%, parametrial white adipose tissue weight by 56%, serum TAG by 52%, TC by 18%, and hepatic TAG by 56%, compared to the HFD.  
- Supplemented fiber diets showed a decrease in BW, adipose weight, TAG, glucose, and insulin levels based on dosage levels. Cecal SCFA concentrations
<table>
<thead>
<tr>
<th>Study</th>
<th>Animals</th>
<th>Diet Details</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brockman et al., (2014)</td>
<td>Wistar rats (48); 6 weeks old; male</td>
<td>1) Normal fat diet (26% energy from fat); HFD (60% energy from fat) w/ 2) 5% cellulose – nonviscous and nonfermentable; 3) 5% guar gum – fermentable and viscous; 4) 5% hydroxypropyl methylcellulose (HPMC) – nonfermentable and viscous</td>
<td>10 wks</td>
<td>- Epididymal fat pad weight of the normal fat, guar gum, and HPMC diets was lower than the cellulose (nonviscous) diet. In addition, fat mass percentage and liver lipid concentrations did not differ among these viscous and normal fat diets. Viscosity played a major role than their fermentable property. - BW and visceral fat of both HFTF and Westernized diet increased compared to the standard chow diet, as well as signs of insulin resistance. Westernized diet w/ cholesterol increased serum leptin, liver weight, relative liver weight/BW ratio, and fibrosis.</td>
</tr>
<tr>
<td>Mells et al., (2015)</td>
<td>C57BL/6J mice (72) 4-5 weeks old; male</td>
<td>Both HF diets have 45% energy from fat w/ 30% of fat from partially hydrogenated vegetable oil: 1) HFD w/ trans-fat (TF) 2) Westernized diet: HFTF w/ 0.2% cholesterol 3) Standard chow diet</td>
<td>16 wks</td>
<td>- Increased compared to the HFD w/0% guar gum (control), but there did not seem to be a link between the different guar gum dosages and metabolic markers.</td>
</tr>
</tbody>
</table>


1.7 Use of Mice Model: C57BL/6

Human trials are costly and time-consuming to perform; therefore animal models are often used to carry out research. In particular, obesity research uses genetic and diet-induced mice models to represent the biological and physiological functions in the human species (Li et al., 2008). Mice are commonly used because of their small size, short gestation period, low maintenance cost, and ease of breeding (Gallaher et al., 2000; Yazdi et al., 2015). Mice even reach sexual maturity faster than any other mammal, which helps with obtaining results to generate potential research findings in a reasonable amount of time. There is an abundance of genetically defined mice strains in research studies that have been previously tested to establish consistent gene functionality (Yazdi et al., 2015). However, genetic mice models are quite ideal in mimicking human biological pathways because genetic alterations in humans with obesity symptoms are not fully understood. It is also still unclear as to how well diet-induced mice models represent human obesity, even though they do provide a broader range of obesity symptoms of increased body weight, adiposity, triglyceride levels, and circulating insulin levels (Li et al., 2008).

Studies have shown that the daily consumption of soluble DF attenuates postprandial rise in blood glucose levels and can help reduce the risk of Type 2 diabetes and cardiovascular disease in humans (Panahi et al., 2007). Feeding a HFD induces a more pronounced weight gain showing characteristics of insulin resistance and impaired glucose tolerance compared to a standard chow diet (26% energy from protein, 63% energy from carbohydrate, and 11% energy from fat). The C57BL/6 mice that consume a HFD (58% fat energy from fat) reflect the human condition of Type 2 diabetes the best, without breeding mice to develop naturally occurring mutations or genetic mutations. In general, this diet-induced obesity (DIO) mouse model is commonly used for studies about cardiovascular disease, diabetes, and obesity (King et al., 2012). The studies described in Table 1.1 use mainly C57BL/6 mice as animal models. All the studies listed use different inclusion levels of DF supplemented in the HFD, different types of DF, or a Westernized diet compared to a standard, control diet. The impact of using a supplemented diet in these studies allow for significant findings pertaining to the changes in obesity parameters.
1.8 Conclusion

Many studies have been conducted on the path of reducing symptoms of obesity and its associated metabolic diseases by dietary fiber supplementation, but there have been many inconsistencies based on the proposed effects of dietary fiber components regarding its source, dose, diet preparation, linkage and molecular size, and even the overall study design. Pharmacology approaches to reducing obesity have only been moderately successful with the consequence of some serious side effects. Dietary fiber supplementation has potential beneficial health effects, but its effects have been complicated or unclear. There still needs to be an in-depth understanding of dietary fibers to help identify effective and preventative dietary approaches in improving symptoms of obesity and its other health risks.
Hypothesis

This study hypothesized that both dietary fibers (konjac glucomannan or oat β-glucan) will affect the digestive physiology and health in high-fat diet induced obese mice by affecting the fat accumulation in the liver, short chain fatty acid production in the colon, and the different hematological parameters.

Objectives

The overall objective of this study was to investigate how two different dietary fibers (glucomannan and oat β-glucan) at varying inclusion levels (1.25, 2.5, and 5%) will affect the digestive physiology and health in high-fat diet (HFD) induced C57BL/6 mice. The specific objectives of this study was to evaluate effect of dietary fibers with different inclusion level on the characteristics of obesity related parameters like body weight gain and liver growth, hepatic histopathology, liver steatosis severity, short chain fatty acid production in the cecum, and serum metabolites of insulin, glucose, triglycerides, and cholesterol levels.
CHAPTER 2: METHODS

2.1 Materials and Methods

The animal study was performed in accordance with The Guide for the Care and Use of Laboratory Animals (Institute of Medicine, 1996) and carried out under protocol #13-1730 approved by the Institutional Animal Care and Use Committee at University of Hawaii at Manoa.

2.2 Animals and diets

Male C57BL/6 mice \( (n = 84) \) at four weeks old were purchased from a commercial supplier (Harlan Laboratories, Indianapolis, IN, USA). All mice were fed a purified HFD (TD.06414) prepared by Teklad Research Diets (Madison, WI, USA) and were transported to the Small Animal Facility (SAF) located at the University of Hawaii at Manoa. During the acclimation period of 2 weeks, the HFD maintained diet-induced obesity in the mice. With the exclusion of blue food coloring, this modified HFD provided a total calorie content of 5.1 kcal/g with approximately 18.4% energy from protein, 21.3% energy from carbohydrates, and 60.3% energy from fat (primarily lard). The diet was composed of (g/kg of diet): lard 310; casein 265; maltodextrin 160; sucrose 90; cellulose 65.5; AIN-93G mineral mix 48; soybean oil 30; AIN-93G vitamin mix 21; L-Cystine 4; dicalcium phosphate 3.4; and choline bitartrate 3. Diet ingredients were obtained from Test Diet (St. Louis, MO, USA), LD Carlson Company (maltodextrin; Kent, OH, USA), Bulk Supplements (dicalcium phosphate; Henderson, NV, USA) and local grocery stores. For the HFD, the analyzed ether extract value was 32.81% and the analyzed gross energy value was 5.89 kcal/g.

The Control diet was based on the American Institute of Nutrition (AIN)-93G diet formulated specifically for the growth performance of rodents (Reeves et al., 1993). This standard diet consisted of 18.8% energy from protein, 65.1% energy from carbohydrates, and 16.4% energy from fat. The formulation energy of GM and OBG diets were modified from AIN-93G by supplementing it with GM and OBG at 1.25%, 2.5%, and 5% inclusion levels. Cellulose was not used because GM and OBG were the test ingredients.
Glucomannan (from *Amorphophallus Konjac*) was sourced from Now Foods (Bloomingdale, IL, USA). It consisted of 100% GM powder that provided 2.5 kcal/g of energy. The OBG was sourced from VDF FutureCeuticals, Inc. (Momence, IL, USA). It consisted of 20% β-glucans that provided 3.47 kcal/g of gross energy. Following Besten et al., (2014) and other previous studies, all dietary treatments were isoenergetically balanced by changing the amount of cornstarch to replace the amount of varying GM and OBG ingredients. All dietary treatments were mixed at SAF during the acclimation period and stored in airtight containers until the start of the study. The analyzed gross energy values for the GM powder and OBG source were 3.53 and 4.22 kcal/g, respectively.

The ingredient listings and composition of diets detailed in Table 2.1 were formulated according to specific experimental properties: 1) standard commercial rodent chow diet (having no GM or OBG, Control), 2) GM at 1.25 %, w/w, (GM1.25), 3) GM at 2.5 %, (GM2.5), 4) GM at 5 %, (GM5), 5) OBG at 1.25 %, w/w, (OBG1.25), 6) OBG at 2.5 %, (OBG2.5), and 7) OBG at 5 %, (OBG5). All diets were given to the mice in powdered form and measured out twice a week for 12 weeks. However, because of the loose powdered form, a substantial amount of food spillage occurred. The powder caught onto their fur or was thrown out of their feed bowl and settled down into the bedding. So, all diets were mixed with water to create a pellet form that started in the second week of study.

2.3 Experimental design and housing

During the acclimation period of 2 weeks, the mice were fed a modified version of the HFD as they recovered from exertion and transportation stress. The mice were maintained under a controlled environment with conditions of temperature at 70-74°F, humidity at 55 ± 5%, and an automatic alternating 12 h light - 12 h dark cycle lighting program. All mice had free access to food and water.

At 6 weeks of age, the mice were allocated into one of the seven dietary treatments ($n = 12$) randomly with 2 mice per cage, which were designated as Control; glucomannan: GM1.25, GM2.5, GM5; and oat β-glucan: OBG1.25, OBG2.5, OBG5. Identification of each mouse was done through ear tagging prior to being housed together (2 mice per cage) in polycarbonate cages with wire lids and small wood chip pellet bedding at the start of the study. Their physical activity and behavior were monitored daily. If there were any signs of abnormality or stress, then it was
noted in a logbook. All mice completed the study except for one mouse from OBG1.25 that died from its cage partner displaying aggressive behavior toward it and withdrawn from the study. Two other mice from GM2.5 and OBG5 showed physical signs of being in a fight and were also removed from the study.

2.4 Body weight measurements

After the acclimation period of the mice being fed the HFD, baseline body weight of each mouse was measured on a standard electronic weighing scale (MP-1000; ACUweigh; Fujian, China). Subsequently, the mice were weighed during the same time once a week using the same weighing scale.

2.5 Mice sacrifice and samples collection

On the last day of the study (Day 84), the mice were sacrificed by CO₂ inhalation under human supervision after 12 hours of overnight fasting. Whole blood was collected by an open approach cardiac puncture technique performed as a terminal procedure following University of Delaware’s Laboratory of Animal Medicine’s Standard Operating Procedure. Briefly, place the mouse on its back and sterilize the chest area with alcohol wipes. With a scalpel, make a clean skin incision to fold back the skin flaps to expose the heart. Insert the 21 gauge 1” needle at a 45° angle to slowly aspirate on the syringe plunger. Withdraw the needle once the needed amount of blood has been collected, then dispose of mouse in biohazard bag and into freezer for future incineration. To prepare the serum sample, the blood was transferred into 1.5 mL Eppendorf tubes to let stand for 30 min at room temperature. Blood serum was isolated by centrifugation at 2400 rpm for 20 min with the required sample amount (200 µL) being pipetted into labeled cryovials. All samples were stored at -80°C until further analysis.

The mice livers were excised, rinsed with double distilled water, and quickly weighed on the same standard weighing scale. A small portion of a liver lobe was immediately fixed in 10% formalin and left overnight at room temperature. The next day, small liver samples were embedded in paraffin and 5µm sections were stained with hematoxylin-eosin (H&E), placed onto microscope slides with cover slips to be left to dry overnight at the Histocore of John A. Burns
School of Medicine, University of Hawaii at Manoa, in preparation for further histopathological analysis.

After locating the cecum along the large intestine, it was excised and rinsed with double distilled water. The cecal content was gently milked into sterile, plastic bags and stored at -80°C for further SCFA analysis.
Table 2.1 Ingredient composition and analyzed nutrient content of experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Glucomannan (GM)</th>
<th>Oat Beta-Glucan (OBG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.25%</td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cornstarch (100% amylopectin)</td>
<td>580.7</td>
<td>568.7</td>
<td>555.7</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Mineral Mix¹</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin Mix²</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>BHT³</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Dietary Fiber: 100% GM</td>
<td>0.0</td>
<td>12.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Dietary Fiber: 20% OBG</td>
<td>0.0</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Analyzed nutrient content, as fed

| Gross Energy, kcal/g⁴            | 4.37  | 4.42 | 4.30 | 4.26  | 4.35 | 4.30 | 4.41 |
| CP, %                            | 18.01 | 18.50| 19.35| 21.52 | 17.50| 17.28| 16.81|
| Ether Extract, %⁵                | 0.94  | 1.68 | 1.35 | 1.15  | 1.46 | 1.38 | 1.74 |

¹AIN-93G provided (per kilogram of diet) (%): 0.51 as calcium; 0.32 as phosphorus; 0.36 as potassium; 0.05 as magnesium; 0.13 as sodium; 0.22 as chlorine; in (ppm): 1.0 as fluorine; 39 as iron; 35 as zinc; 11 as manganese; 6.0 as copper; 0.0 as cobalt; 0.21 as iodine; 1.0 as chromium; 0.14 as molybdenum; 0.24 as selenium.

²AIN-93G provided (per kilogram of diet): 4.0 IU/g as Vitamin A; 1.0 IU/g as Vitamin D-3 (added); 81.6 IU/kg as Vitamin E; 0.29 ppm as Vitamin K (as menadione); in (ppm): 6.1 as thiamin hydrochloride; 6.7 as riboflavin; 30 as niacin; 16 as pantothenic acid; 2.1 as folic acid; 5.8 as pyridoxine; 0.2 as biotin; 1.250 as choline chloride; 0.0 ascorbic acid; 29 mcg/kg as Vitamin B-12.

³Butylated hydroxytoluene

⁴Gross energy value of GM and OBG were 3.53 and 4.22 kcal/g, respectively.

⁵Ether extract value of GM and OBG were 0.10 and 1.54 %, respectively.
2.6 Nutrient profile of diets

All 7 experimental diets (including the HFD) were analyzed for their basic nutrients composition: gross energy, crude protein, and crude fat (ether extract) using standard methods (AOAC, 2006). Gross energy was determined by using a bomb calorimeter (Model 6200; Parr Instrument Company, Moline, IL, USA). Crude protein was determined by a Leco Analyzer (Method 968.06; Model FP-528; Leco Corp., St. Joseph, MI, USA) and crude fat was analyzed (AOAC 920.39; using Soxhlet apparatus and petroleum ether).

2.7 Analysis

2.7.1 Biochemical analysis of blood serum samples

All samples were analyzed in food-deprived mice with the serum obtained from the heart. Serum insulin concentrations were determined by ELISA assay (Millipore, St. Charles, Missouri, USA). Serum glucose concentrations were measured using a commercial kit (Randox Laboratories Limited, UK). Serum triglycerides and total cholesterol concentrations were measured using commercial kits (Pointe Scientific, Inc., Canton, MI, USA). These metabolites were determined by an autoanalyzer (Cobas Mira Plus CC, Roche Diagnostics, Basel, Switzerland) at the Cancer Center, University of Hawaii at Manoa.

2.7.2 Histopathological analysis of liver samples

The H&E stained sections (5μm) of liver tissue were examined under a light microscope at a medium power field (200x) to evaluate the severity of steatosis in the small liver lobe samples. Hepatocytes were classified as having microvesicular, mediovesicular, and macrovesicular steatosis pertaining to their lipid droplet size (Trak-Smayra et al., 2011). The severity levels ranged from + (0-33%) as mild, ++ (33-67%) as moderate, or (67%-100%) as severe steatosis (Table 3.2). Each severity level of steatosis was averaged out for each mouse in 5 randomly chosen zones per dietary treatment (n=4-6). Fatty liver was determined by its pink coloration later confirmed by histological analyses showing the presence of microvesicular fat in >5% of the hepatocytes per slide. These samples were also evaluated on the percentage of steatosis within the chosen zones demonstrating lipid accumulation (Trak-Smayra et al., 2011).
2.7.3 Short chain fatty acid analysis of cecal samples

Short chain fatty acids (acetate, propionate, butyrate) of the cecum were analyzed using a gas chromatography method modified from Stewart et al. (2010). Briefly, a 150 mg cecal sample was pooled from a total of 3 mice from the same dietary treatment due to a limited amount of cecal content. In a centrifuge tube, the sample was mixed with 400 µl of sulfuric acid (50%), 3 µl of ethyl butyrate as the internal standard, and double deionized water to create a total volume of 3 ml. Samples were gently vortexed at room temperature, then centrifuged at 4200 rpm at 4°C for 15 min. The supernatant (1.5 mL) was transferred into 1.5 mL Eppendorf tubes and centrifuged again at 3000 rpm at 25°C for 5 min. The supernatant (1mL) from that was loaded into a GC vial, capped, and placed in the freezer for future analysis on the GC machine. Analysis was conducted using a Stabilwax DA column (30 m, 0.53 mm internal diameter, 1-µm film thickness; Restek, Bellefonte, PA., USA). Helium was the carrier gas (24 ml/min). Hydrogen and air flow rates were 34 and 410 ml/min, respectively. The oven temperature was maintained at 100°C for 2 min and then increased to 120°C at 60°C per minute. Inlet and detector temperature were both at 200°C.

Standards for propionate and butyrate were prepared using a SCFA mix, deionized water, sulfuric acid (50%), and ethyl butyrate as the internal standard, as shown in Table 2.3. The SCFA mix contained 10 organic free fatty acids, but propionate, and butyrate were of interest. Instead of using a SCFA mix for the acetate standard, a stock solution of acetate was used, as shown in Table 2.2. They each represented a standard curve for a known concentration. After analyzing the cecal samples through the GC machine, their chromatograms were compared to the standard curves.
2.8 Statistical Analysis

All data on body growth performance, blood serum metabolites, steatosis, and SCFA production were analyzed as a completely randomized design with 6 treatments arranged factorially with fiber type, inclusion level, and their interactions as the main effects. Statistical analysis was conducted using MIXED procedure of SAS version 9.2 (SAS INC., Cary, NC, USA), with mice being the experimental unit for all variables studied. Means were separated by the Tukey’s post hoc test. All data were expressed as means with their standard errors. Differences were considered significant at $P<0.05$. 

<table>
<thead>
<tr>
<th>Concentration (µmol/ml)</th>
<th>100mM stock Acetate solution (µl)</th>
<th>Sulfuric Acid (µl) (50%)</th>
<th>Internal Standard (µl)</th>
<th>ddH2O (µl)</th>
<th>Total Volume (mL)</th>
</tr>
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<tbody>
<tr>
<td>1.0</td>
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<td>855.7</td>
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<tr>
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<tr>
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<td>1</td>
<td>845.7</td>
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<table>
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<th>Concentration (µmol/ml)</th>
<th>VFA standards (µl)</th>
<th>Sulfuric Acid (µl) (50%)</th>
<th>Internal Standard (µl)</th>
<th>ddH2O (ml)</th>
<th>Total Volume (mL)</th>
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<tr>
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<td>1500</td>
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<tr>
<td>6.0</td>
<td>1800</td>
<td>400</td>
<td>3</td>
<td>0.80</td>
<td>3</td>
</tr>
</tbody>
</table>
CHAPTER 3: RESULTS

3.1 Effect of dietary treatments on body weight and liver growth

Over the 12-week study, the body weight of all mice increased from when they started consuming the HFD at 6 weeks old. After 8 weeks, mice fed the GM diets had significantly lower body weight gain (P<0.0004) than the mice fed the OBG diets, even though the body weight difference varied somewhat between each of the experimental series (Table 3.1). This trend did not follow between week 8 to week 12. Although, the overall study of 12 weeks showed that the body weight significantly reduced in mice fed GM diets compared to the OBG diets (P< 0.0045; Table 3.1). Regarding the average liver weight of the mice, there was no significant effect (P>0.05) of different fibers, their inclusion levels, or any interaction. However, there was an interaction (P<0.046) between fibers and their inclusion levels on the average relative liver weight, being significantly lower in GM5 and all OBG levels diets (Table 3.1).

3.2 Effect of dietary treatments on serum metabolites

All serum metabolites (insulin, triglyceride, cholesterol, and glucose) were analyzed by commercial kits and by an autoanalyzer. Two mice from the Control diet and one mouse from OBG5 did not provide enough sample for the serum triglyceride, cholesterol, and glucose analysis. For all four metabolites, one mouse died in OBG1.25 from physical, aggressive behavior from its cage partner. These four mice were excluded from the biochemical analysis.

At the end of the study, mice that demonstrated unusually high or low serum metabolite concentrations were considered outliers and excluded from the statistical analysis. In the serum insulin assay (ran in duplicate), a total of 12 outliers had these extreme values: Control diet at 175.37 pmol/l; GM1.25 at 90.87 and 1017.11 pmol/l; GM5 at 72.28 and 722.82 pmol/l; OBG1.25 at 810.94 and 1136.72 pmol/l; OBG2.5 at 1093.70, 1267.00, and 1379.21 pmol/l; and OBG5 at 1146.70 and 1203.50 pmol/l (n=70). For the serum triglyceride assay, GM5 had one outlier with a high value at 2.89 mmol/l (n=79). There were no outliers in the serum cholesterol assay (n=80), but for the serum glucose assay, OBG5 had two outliers at 5.24mmol/l and 7.11 mmol/l, respectively (n=78).
The main results showed that mice fed the OBG diets had significantly lower serum triglyceride concentrations compared with those fed the GM diets. Meanwhile, the mice fed GM2.5 and GM5 actually showed an increase in serum triglyceride concentrations ($P < 0.02$; Table 3.2). There were no significant differences found for serum insulin, cholesterol, and glucose concentrations in between GM or OBG diets ($P > 0.05$; Table 3.2).

3.3 Effect of dietary treatments on histopathology of liver

A pathological examination of the H&E stained liver cross-sections was performed through light microscopy to evaluate the fat droplet size within hepatocytes. All mice demonstrated marked steatosis except for 1-2 mice per dietary treatments that showed no evidence of fatty liver disease after the 12-week study because there was no lipid accumulation in the hepatocytes, while others in the same dietary treatments showed some levels of lipid accumulation. These mice were excluded from the evaluation of steatosis severity and the statistical analysis on steatosis percentage.

All steatosis types ranging from mild, moderate, and to severe were visible through the light microscope observation within each dietary treatments (Figure 1). However, it was noteworthy to mention several observational trends. The steatosis evaluation showed both GM1.25 and OBG1.25 developing severe microvesicular fat compared to the other diets with only GM5 showing a noticeable lipid reduction by 60% compared to the Control diet (Figure 1). In addition, the effect of the GM diets slightly decreased the occurrence of mediovesicular fat, while it was bit more prevalent in the OBG diets.

Interestingly, the effect of both GM and OBG diets decreased the occurrence of macrovesicular fat altogether compared to the Control diet. In particular, GM5 was the only diet that reduced the severity of all steatosis levels. Regarding percent steatosis, the mice the OBG diets showed a significantly lowering effect compared to the mice fed GM diets ($P < 0.001$; Table 3.1). There was a significant interaction between the fibers and their inclusion levels. More specifically, a distinguishable steatosis percent reduction of 68% came from GM5 compared to the Control diet. An Axio Scope at 400x took representative photomicrographs from the sectioned liver slides (Figures 2-5).
3.4 Effect of dietary treatments on short chain fatty acid production

Cecal content was pooled together from a total of 3 mice per dietary treatment, creating 4 replicates each. The distribution of SCFA generated the highest pool of acetate, propionate, and butyrate among the dietary treatments, presented in Table 3.4. These results showed acetate (P<0.0097) and propionate (P<0.0235) concentrations in the cecal content being significantly higher in the mice fed GM diets compared to the OBG diets (Table 3.4). There were no significant effects of the two fiber diets, their inclusion level, or any interaction for the butyrate concentrations.
<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GM</th>
<th>OBG</th>
<th>SEM (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.25%</td>
<td>2.5%</td>
<td>5.0%</td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>Growth Performance, g</td>
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<tr>
<td>Initial BW¹</td>
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<td>28.8</td>
<td>29.6</td>
<td>29.6</td>
<td>0.90</td>
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<tr>
<td>Final BW¹</td>
<td>38.0</td>
<td>35.4</td>
<td>37.2</td>
<td>36.8</td>
<td>0.85</td>
</tr>
<tr>
<td>Body Weight Gain, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: 0 to 4 wks</td>
<td>3.3</td>
<td>1.4</td>
<td>2.4</td>
<td>1.5</td>
<td>0.38</td>
</tr>
<tr>
<td>2: 4 to 8 wks</td>
<td>3.0</td>
<td>1.2</td>
<td>2.5</td>
<td>2.4</td>
<td>0.49</td>
</tr>
<tr>
<td>3: 8 to 12 wks</td>
<td>3.0</td>
<td>4.1</td>
<td>2.8</td>
<td>3.4</td>
<td>0.56</td>
</tr>
<tr>
<td>4: 0 to 12 wks</td>
<td>9.3</td>
<td>6.7</td>
<td>7.7</td>
<td>7.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Liver Weight (g)</td>
<td>1.84</td>
<td>1.61</td>
<td>1.51</td>
<td>1.42</td>
<td>0.06</td>
</tr>
<tr>
<td>Relative Liver Weight²</td>
<td>4.82</td>
<td>4.56</td>
<td>4.07</td>
<td>3.85</td>
<td>0.14</td>
</tr>
<tr>
<td>% Steatosis</td>
<td>93.8</td>
<td>95.0</td>
<td>80.8</td>
<td>30.0</td>
<td>0.66</td>
</tr>
</tbody>
</table>

¹Body weight
²Relative to final live body weight, %

a-d Mean values with unlike letters were significantly different (P<0.05) after 12 weeks on the dietary treatments
<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GM 1.25%</th>
<th>GM 2.5%</th>
<th>GM 5.0%</th>
<th>OBG 1.25%</th>
<th>OBG 2.5%</th>
<th>OBG 5.0%</th>
<th>SEM (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=10</td>
<td>n=12</td>
<td>n=11</td>
<td>n=12</td>
<td>n=11</td>
<td>n=12</td>
<td>n=11</td>
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<td></td>
</tr>
<tr>
<td>Insulin, pM (n=70)</td>
<td>555.1</td>
<td>573.4(^{1})</td>
<td>365.7</td>
<td>430.5(^{2})</td>
<td>454.0(^{3})</td>
<td>569.3(^{4})</td>
<td>423.4(^{5})</td>
<td>74.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Triglyceride, mM (n=79)</td>
<td>0.80</td>
<td>0.70</td>
<td>0.89</td>
<td>0.93(^{2})</td>
<td>0.61</td>
<td>0.62</td>
<td>0.63(^{5})</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol, mM (n=80)</td>
<td>5.1</td>
<td>4.6</td>
<td>3.9</td>
<td>3.8</td>
<td>4.0</td>
<td>4.3</td>
<td>3.8</td>
<td>0.22</td>
<td>0.63</td>
</tr>
<tr>
<td>Glucose, mM (n=78)</td>
<td>18.4</td>
<td>16.4</td>
<td>16.7</td>
<td>15.9</td>
<td>14.3</td>
<td>15.4</td>
<td>15.6</td>
<td>0.88</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^{1}\)n=10, excluding two mice that did not provide enough sample
\(^{2}\)n=11
\(^{3}\)n=9, excluding the mouse that died
\(^{4}\)n=9
\(^{5}\)n=10
Figure 1. Histopathological features of the hepatocytes in dietary treatments (0-1: < 33% mild; 1-2: 33-67% moderate; 67<100% severe)
<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GM</th>
<th>OBG</th>
<th>SEM (n=12)</th>
<th>P value</th>
<th>Fiber Level</th>
<th>Fiber × Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.25%</td>
<td>2.5%</td>
<td>5%</td>
<td>1.25%</td>
<td>2.5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>317.3</td>
<td>441.3</td>
<td>438.0</td>
<td>365.3</td>
<td>280.0</td>
<td>274.7</td>
<td>304.0</td>
</tr>
<tr>
<td>Propionate</td>
<td>6.13</td>
<td>6.79</td>
<td>6.73</td>
<td>6.49</td>
<td>6.34</td>
<td>6.12</td>
<td>6.39</td>
</tr>
<tr>
<td>Butyrate</td>
<td>3.78</td>
<td>5.61</td>
<td>5.72</td>
<td>5.44</td>
<td>4.57</td>
<td>3.39</td>
<td>5.21</td>
</tr>
</tbody>
</table>

*1 Pooled SEM (3 mice × 4 replicates per dietary treatment)*
Figure 2. Representation of microvesicular steatosis (H&E, x200)

Figure 3. Representation of mediovesicular steatosis (H&E, x200)
Figure 4. Representation of macrovesicular steatosis (H&E, x200)

Figure 5. Representation of rare fat in hepatocytes (H&E, x200)
CHAPTER 4: DISCUSSION

4.1 Experimental Diets

All dietary treatments were prepared isoenergetic in this study with the energy value of ingredients provided by suppliers. The dietary treatments at varying inclusion levels had either the fiber type GM or OBG except for the Control diet that was prepared fiber-free. Choosing this control diet to be fiber-free was necessary in order to study the effects of just the two DFs instead of possibly obtaining effects from another source of DF (Jakobsdottir et al., 2013). The mice were fed the HFD (60% energy from fat) during the acclimation period of only 2 weeks, and then placed on the experimental diets for 12 weeks. Some obesity symptoms could not have possibly been presented this early compared to other research studies that had a longer duration of a study. For example, research conducted by Chen et al. (2011) studied the effects of a Westernized diet in the same mice model for 17 weeks, while Isken et al. (2010) studied the long term effects of soluble vs. insoluble DF in C57BL/6 mice for a duration of 45 weeks. In contrast, research conducted by Jakobsdottir et al. (2013) showed effects of both low-fat diet (LFD) and HFD for 2, 4, and 6 weeks on SCFA production patterns and metabolic risk factors. Interestingly, there was opposing results depending on the experimental period. Choosing the 12-week period for this present study was suitable enough to demonstrate metabolic risk factors based on the outcome of previous studies.

Elaborating on the research performed by Chen et al. (2011), this study included a HFD at 60% energy from fat, a HFD that resembled a Western-style diet composed of lower levels of calcium, vitamin D₃, folic acid, choline bitartrate, and fiber, as well as a LFD at 10% energy from fat. Their findings in the Western-style diet demonstrated not only more of a body weight gain in the C57BL/6 mice, but also severe metabolic syndrome risk factors compared to the HFD. Incorporation of this Western diet with its lack of certain nutrients represented the major dietary risk factors humans faced in the modern day life. It would be interesting to have a study that includes both a LFD and HFD to showcase a spectrum of metabolic values, as well as with varying levels of supplemented DF in the HFD.
4.2 Mice Model

Monogenic mice models are most commonly used in Type II diabetic research because of their defective leptin protein signal. These models include Lep\textsuperscript{ob/ob} mice that lack leptin, as well as, Lepr\textsuperscript{db/db} mice and Zucker diabetic fatty (ZDF) rats that have a mutation in the leptin receptor. These monogenic animals are obese models closely linked to the development of type II diabetes characterized by impaired blood glucose concentrations, while polygenic animal models of obesity show the interplay between many genotypes. Depending on the specific area of interest in regards to obesity or diabetes, monogenic or polygenic animal models are chosen. However, obesity and diabetes in humans are rarely caused from genetic mutations let alone just one mutation, and rather induced by environmental factors leading to a more realistic onset of obesity (Jones, 2013). Therefore, C57BL/6 mice strain was developed to be susceptible to high fat feeding and inducing risk factors leading to obesity, insulin resistance, and diabetic complications (King et al., 2012).

Other monogenic animal models (db/db mice and ZDF rats) used in research studies have helped investigate the antidiabetic effects of glucose and insulin tolerance, while assessing the effect of a certain gene expression in liver cancer cells, as well as the liver adipose tissue weight (Wolfram et al., 2006). Another study compared leptin-deficient ob/ob and db/db mice models to examine liver pathology in a standard chow diet compared to a HFD. They focused on steatosis, necroinflammation, apoptosis, and fibrosis in hepatocytes (Trak-Smayra et al., 2011).

4.3 Body weight gain and liver growth

In present study, the mice consumed a HFD that had a substantial increase in calories from fat (around 60.3% of energy from lard), then switched to the standard AIN-93G diet (on a caloric basis: 65.1% carbohydrates, 18.8% protein, and 16.4% energy from fat) for 12 weeks (Reeves et al., 1993). This HFD purposefully increased their body weight to induce associated obesity risk factors. The OBG powder only provided 20% of β-glucans compared to the pure 100% konjac GM powder, where the amount of OBG powder used for OBG1.25, OBG2.5 and OBG5 was higher than the GM diets. This was done to have the same inclusion levels (1.25%, 2.5%, and 5%) between the two DF treatments. These diets were analyzed and were found to have similar gross energy values (Table 2.1). This study showed that the mice consuming the
GM diets for 12 weeks had an overall significant reduction in body weight gain (BWG) compared to the OBG diets.

Many studies have been conducted to demonstrate the effects of DFs on body and liver weight performance in mice. To discuss a few, research conducted by Gallaher et al. (2000) showed the Wistar rats fed GM, chitosan (CH; a derivative of chitin), or GM + CH diets having a significantly lower body weight and reduced liver weight than the rats fed the control (cellulose) diet. Daily fecal fat excretion was significantly higher in CH + GM and CH compared to GM and control. In another study, C57BL/6 mice were fed a standard rodent diet (AIN-76A w/ 65% corn starch), a HFD, and three other HFD with β-glucans at different molecular weights. The experimental HFD supplemented with β-glucans significantly reduced BW of mice, but did not seem to affect the serum lipid profile (Bae, et al., 2009).

This trend of reduction in body and liver weight was also found in research conducted by Zhang et al. (2012) with C57BL/6J mice being fed 2% or 5% Salecan, a soluble, viscous β-D-1,3-glucan polysaccharide as the main backbone structure with some α-(1,3) linkages supplemented in a HFD (64% energy from fat). The differences in the type of linkages and the chemical structure impacted solubility and biological functions with Salecan’s structural composition being slightly different from OBG’s β-(1,4) and β-(1,3) linkages.

Regarding the liver weight of the mice, there was no significant effect of the different fibers, their inclusion levels, or any interaction. Although, there was an interaction between fibers and their inclusion levels based on relative liver weight. All OBG diets were had significantly lowered the average relative liver weight compared to the GM diets and Control diet with the exception of GM5 being just as significant. This liver weight reduction was also found by Zhang et al. (2012) that studied with Salecan treatments. It has been suggested that Salecan and other β-glucans could help reduce the rate of fat digestion and absorption by protecting against fat accumulation in the liver.

4.4 Serum metabolites

This present study showed no significant differences in serum insulin, cholesterol, and glucose concentrations between GM and OBG diets. Immerstrand et al. (2010) stated that the difference in viscous properties of the varied β-glucan molecular weights did not play a major role in their cholesterol-lowering effects. Although, there was a significant plasma cholesterol-
lowering effect from the different β-glucan molecular weights (<10, 21, 56, 241, 1311 kDa) compared to the control (cellulose) diet. However, sterols, lipids, or other oat components could have indirectly led to this, which would also overall have no effect on cholesterol-lowering properties.

More specifically, a previous study mentioned above by Gallaher et al. (2000) had the liver cholesterol concentrations in the Control diet being approximately twice the values in the GM, CH, and GM + CH dietary treatments, while serum cholesterol and glucose concentrations did not show any significant effect with the DF supplementation. This could be because the amount of cholesterol used in the experiment was considerable low. Another reason cholesterol levels were not significant was described in a research study by Hundemer et al. (1991) that stated soybean fiber and rice bran were the only two DF sources that significantly lowered total serum cholesterol than the fiber-free control. More specifically, the soybean fiber diet significantly lowered the blood cholesterol than the mixed bran. This suggested using a different DF source other than OBG could possibly improve the blood and liver cholesterol.

This present study showed the effect of OBG diets reducing the serum triglyceride concentrations significantly compared with those of the GM diets and the Control diet. Bae et al. (2009) also had similar findings that suggested although some biological functions are improving serum triglycerides, other factors could possibly be contributing to this effect and not just the OBG diet. Overall, this is an indication that the consumption of DF has some beneficial metabolic health effect.

Tian et al. (2013) investigated the effect of β-glucans in highland barley supplemented in a HFD at either 2% or 4%, in addition to a HFD and control diet. Results showed that highland barley diets helped reduce serum glucose and serum lipids, which led to improving insulin sensitivity. In another study, Gao et al. (2009) examined the role of 5% wt/wt of sodium butyrate being incorporated into a HFD compared to a plain HFD in C57BL/6 mice. These researchers found that the butyrate supplementation in HFD had an increase in plasma butyrate concentration, while the blood lipid profiles (triglycerides, cholesterol, and totally fatty acids) decreased. The decrease in fat accumulation suggested that it helped prevent the development of insulin resistance. The role of butyrate promoted energy expenditure and mitochondrial functions, which may have contributed to the prevention and treatment of diet-induced obesity in mice models.
4.5 Histopathology of liver

Hepatic steatosis (NAFLD) has been one of the main metabolic risk factors of obesity. Examining the amount and type of steatosis severity levels in the mice livers has helped show the interaction between fat accumulation within the hepatocytes and obesity related health issues. This part of the study investigated the percentage of steatosis in hepatocytes with one or numerous lipid vacuoles evaluated in H&E stained sections by a light microscope. Both GM1.25 and OBG1.25 diets established microvesicular fat as being more severe than the other diets at inclusion levels of 2.5% and 5%. The GM diets showed milder mediovesicular steatosis compared to the OBG diets. Interestingly, macrovesicular steatosis was greatly reduced in both GM and OBG diets compared to the control diet.

Mells et al. (2015) conducted a study using male C57BL/6 mice that consumed a Westernized diet containing 0.2% cholesterol with 45% of its energy from fat (30% of the fat in the form of partially hydrogenated vegetable oil) being compared to a diet without cholesterol for 16 weeks. It was very crucial for the researchers to compose a diet reflective of what the average Americans consumed on a daily basis. Interestingly, they added high fructose corn syrup to the available water to mimic the human consumption of soft drinks. They investigated how the C57BL/6J mice would be affected by the Westernized diet with cholesterol by looking for indicative signs of metabolic syndrome and the development of nonalcoholic steatohepatitis (NASH) that can come from NAFLD. Common signs of NASH were hepatic lesions with mixed lobular inflammation, degeneration of hepatocytes, and pericentral deposition of fibrillar collagen, and the development of Mallory bodies. Their results showed a significant increase in liver weight, relative liver weight/body weight (resulted in this present study), and hepatic fibrosis.

Hepatic fibrosis being on the severe end of the NAFLD spectrum encompasses steatosis with inflammation and necrosis with or without fibrosis. Gentile & Pagliassotti (2008) emphasized in a review on the development and progression of NAFLD that it is important to recognize the association between circulating fatty acid composition and liver dysfunctions in animal models susceptible to the NAFLD symptoms to determine how this can relate to humans.

Trak-Smayra et al. (2011) investigated liver pathology in ob/ob and db/db mice fed a standard chow diet or a HFD for either one or three months. Severe steatosis was seen in ob/ob mice compared to the db/db mice and it was mostly microvesicular fat in the hepatocytes. This
seemed to be directly associated to apoptosis being observed in the ob/ob mice once they consumed the HFD, which suggested to have come from mitochondrial abnormalities. It would have been helpful to assess more information from the liver regarding necroinflammation, apoptosis, and fibrosis. These assessments past the beginning stages of NAFLD could have led to a better understanding of the dietary treatments affecting the biological functions of the liver enough to conclude a few significant interactions.

For further biochemical analysis, it would have been interesting to determine the concentration of hepatic triglycerides. This quantification could accurately demonstrate the importance on varying hepatic triglyceride concentrations. Zhang et al. (2013) with the Salecan treatment performed this analysis and found decreased fat absorption, improved glucose tolerance, and reduced total cholesterol concentrations. While there were no significant differences found for serum glucose, insulin, and cholesterol concentrations in between GM or OBG diets.

The mice fed GM5 and OBG5 significantly lowered the percent steatosis within their respective fiber type, while GM1.25 demonstrated the highest percent steatosis of all the dietary treatments. Overall, the mice fed the OBG diets had significantly lower percent steatosis in hepatocytes compared to the GM diets. This portion of the study demonstrated a significant interaction between the fibers and their inclusion levels.

As mentioned previously, Chen et al. (2011) implemented a Westernized HFD that lacked adequate amounts of nutrients. In particular, there was a deficiency in choline, which has been known to promote hepatic steatosis. Without choline, excess triglycerides remained in the liver, promoted liver cell apoptosis, and hepatic steatosis became recognizable. Normally, choline has aided biological functions like lipid transportation and metabolism. Therefore, instead of comparing different inclusion levels between two DFs, it might have been interesting to see the effects of varying fat levels, especially one that paralleled a modernized human diet, a Westernized diet. Fortunately, choline chloride was composed in all dietary treatments in this present study.
4.6 Short chain fatty acid production

Isken et al. (2008) supplemented soluble guar gum fiber (10% w/w) or insoluble cereal fiber (10% w/w) into an isoenergetic and macronutrient matched high-fat Westernized diet in C57BL/6J mice for 45 weeks. Mice that consumed the soluble guar gum fiber showed less energy lost through feces excretion and a marked increase in SCFA production. This suggested consuming the soluble guar gum DF had a contribution to energy intake. However, the long-term supplementation with this soluble guar gum led to obesity symptoms displayed in the mice, whereas the insoluble cereal fiber significantly improved insulin sensitivity and significantly lowered body weight gain.

Those findings were also shown in this study where the DF of either GM or OBG had no significant influence on the analyzed amount of butyrate concentration. With this SCFA being considered a substrate for colonocytes, it has been implied that other unknown mechanisms could likely be involved. Jakobsdottir et al. (2013) found a decrease in butyrate concentration that was associated with an increase in succinate concentration. It was suggested to have inhibited the motility of the large intestine and allowed water secretion from the small intestine to happen over time in mice fed a HFD. Inagaki et al. (2007) showed succinate concentration inhibiting the epithelial cell growth rate in the colon. Therefore, it is very important to utilize an adequate supplementation of DF in the treatment and to understand the type of DF used because the production of some SCFAs in the colon may directly or indirectly change the outcome of physiological health.

More importantly in this study, the acetate and propionate concentrations significantly increased in mice fed GM diets compared to the OBG diets. Immerstrand et al. (2010) studied the effect of different molecular weights of β-glucan on SCFA concentrations in the cecum and found all processed oat bran had significantly increased propionate concentrations compared to their control diet with no correlation to the molecular weight of each β-glucan. However, they noted that the ratio of (propionate + butyrate)/acetate increased with heavier molecular weight of the β-glucans. Similarly, Besten et al. (2014) supplemented different inclusion levels of guar gum in a HFD (0, 5, 7.5, or 10%) to look into the changing SCFA concentrations or mechanisms in relation to markers of the metabolic syndrome. All the guar gum supplemented diets lowered the metabolic markers in body weight, adipose weight, plasma and liver triglycerides, and glucose and insulin levels. However, there did not seem to be a relationship between the SCFA
concentrations and the varying inclusion levels on the metabolic syndrome markers. It was suggested that it was the in vivo SCFA uptake fluxes by the host that significantly showed any beneficial effect. Even with this seemingly conclusive statement, it becomes complicated because some disadvantages to this method are a decrease in gut microbiota diversity during isolation, accumulation of SCFAs during fermentation because there are no host uptake mechanisms, and the uptake fluxes by the host are not known. This study suggests that SCFAs positively impact biological functions, but as to what particular pathway or mechanism establishes these beneficial effects are still not clear.

4.7 Conclusion

Glucomannan and oat β-glucan at specific inclusion levels exert significant effects on relative liver weight and percent steatosis in the high-fat diet-induced obese mice model. While oat β-glucan had lower serum triglyceride concentrations, glucomannan had a lower severity in mediovesicular fat, in addition to higher acetate and propionate concentrations. Together, both dietary fibers decreased the severity of macrovesicular fat in the hepatocytes. Thus, supplementing a diet with an adequate amount of specific dietary fiber in high-fat diet-induced obese mice may improve obesity related health issues.
BIBLIOGRAPHY


