The effects of yeast beta-glucan on diabetes, liver cholesterol and fatty liver in rodents

A dissertation

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Introduction

Diabetes mellitus has become a nationwide health problem, and the prevalence of diabetes has increased at an alarming speed. The most recent national diabetes statistic report, released in 2014, jointly produced by the Centers for Disease Control and Prevention (CDC), National Institutes of Health (NIH), and American Diabetes Association (ADA), shows that 29.1 million Americans had diabetes in 2012, which consists of 9.3% of the population. In 2012, 1.7 million of patients are new cases from the last year (1). Besides the noticeable developing speed, diabetes also brought tremendous pain to diabetic patients and huge stress to the medical system. In 2010, Diabetes was the 7th leading cause of death in United States. About $245 billion were spent on diagnosed diabetes patients in 2012 in the United States (1). Patients with diabetes also suffer from many complications, such as hypertension, dyslipidemia, cardiovascular disease, heart attack, stroke, blindness and kidney disease (1).

According to the nutrition guidelines for diabetes patients, published by American Diabetes Association, low glycemic index foods that are rich in fiber are highly encouraged. U.S. Department of Agriculture (USDA) recommended 14 g of fiber per 1000 kcal calorie intake, which is 25-38 g fibers per day per adult and at least half of the grains should be whole-grains (2). Diabetes patients are suggested to at least achieve the fiber intake goal for the general population (2).

The American average dietary fiber intake was 15.9 g per day per adult

in 2007 to 2008 (3), which is much lower than the intake recommended for diabetes. Since the late 1970s, dietary fibers have been shown to have many positive metabolic effects for diabetes patients (4). Over the past three decades, scientists have conducted many studies investigating the mechanisms by which dietary fiber improves diabetes. Many metabolic effects were confirmed to be associated with intake of different types of dietary fibers, including increased satiety and decreased appetite (5), improved insulin sensitivity (6, 7), lower serum cholesterol (8), selective stimulation the growth or activity of gut microflora, decreased inflammatory markers and enhanced intestinal fermentation to produce short chain fatty acids in gut (9, 10). However, dietary fibers have different physical and chemical characteristics, which may play different roles in improving diabetes. Viscous fibers are more likely to be associated with cholesterol lowering and improved glycemic control, while fermented fibers are believed to be more beneficial in promoting a healthy intestinal microflora (11). Insoluble fibers are thought to improve insulin sensitivity (12). Consumption of a variety of fiber-containing foods is encouraged for diabetics, to decrease postprandial glycemic response and achieve a balanced diet with all food groups (2). Foods naturally high in fiber usually have multifarious fibers of varying proportions. Consequently, in order to understand the action of a specific fiber type, it is easier to study a specific fiber rather than a food containing various types of fibers on diabetes.

One of the most controversial fiber types is beta-glucans, which can be obtained from either plant sources (oat, barley) or fungal sources (yeast, mushroom). Beta-glucans is an important component of the cell wall in oats, barley, yeast, and fungi (13). Beta-glucans from oats and barley will increase the viscosity of food and the intestinal environment (14). The beta-glucans linkages are different in plants and yeasts, so beta-glucans from different sources have different physical properties (13) and may lead to different health benefits. Many studies have examined the mechanism of action of plant-based beta-glucans in regards to its ability to improve glycemic control and produce blood lipid lowering (8, 14-18). Relative to oat and barley derived beta-glucans, however, yeast-derived beta-glucans have received much less attention. One advantage of yeast-derived beta-glucans is that they are easier to isolate in higher purity, and they are resistant to heat and pH changes (13). If the yeast beta-glucans have the same benefits for diabetics as oat and barley derived beta-glucans, they may be easier for diabetic patients to take yeast beta-glucans as supplements to diabetic therapy.

Previous studies that concluded that yeast beta-glucans have the same effects as plant beta-glucans on lowering serum lipid and controlling blood glucose (13). However, given the different structures of cereal and yeast beta-glucans, we felt this work needed to be replicated. We hypothesized that yeast beta-glucans may improve diabetes by either lowering the fasting

blood glucose or reducing the postprandial glycemic response. Furthermore, we hypothesized that yeast beta-glucans may reduce hepatic lipid accumulation and hepatic cholesterol concentration. Therefore, this study was designed to determine the effects of consuming yeast beta-glucans on: (1) the glycemic control of diabetes (fasting blood glucose, proportion of glycated hemoglobin and postprandial glycemic response) and (2) hepatic lipid accumulation and hepatic cholesterol concentration. Further, in order to understand which characteristic of yeast beta-glucans might be responsible for the effects found, we determined small intestinal contents viscosity after a meal containing yeast beta-glucans and fermentation with the yeast beta-glucans. We utilized an animal model of diabetes, in which diabetes was induced using the diabetogenic agent, streptozotocin (STZ).

Chapter 1: Literature Review

I Diabetes

Diabetes is a metabolic disease characterized by hyperglycemia that is caused by defects in insulin secretion, insulin resistance, or both (19). The current diagnosis criteria for diabetes is a fasting plasma glucose concentration of 7.0 mmol/L (126 mg/dL) or above, or a blood glucose concentration of 200 mg/dL or above two hours after an oral glucose tolerance test (OGTT), or a casual blood glucose of 200 mg/dl or above (20). Long-term hyperglycemia may impair multiple organs including kidney, liver and the nervous system. Although no evidence indicates that any drug or other treatment can cure diabetes completely (21), some treatments are found to be effective in controlling blood sugar in diabetes or slowing down the process of organ damage (21). Management of diabetes includes hypoglycemic agents, weight loss, regular physical activity, and healthy eating habits (1).

a. Type I and type II diabetes

The most two common forms of diabetes are type Ⅰ diabetes and type Ⅱ diabetes. Type Ⅰ diabetes, which is caused by pancreatic β-cells destruction, which comprises about 5% to 10% of diabetics (19). Type Ⅰ diabetes is an autoimmune disease and has a strong relationship with inherited genetic components (22). The HLA locus is thought to be the most dominate loci contributing to the risk of type Ⅰ diabetes (23). An exaggerated

immune response induces an autoimmune attack on pancreatic β-cells, which causes β-cells damage in the pancreas, resulting in an absolute deficiency of insulin secretion (24). The average age of onset of type I diabetes is much younger than type II diabetes, and the factors that trigger the onset of type I diabetes still remain unknown (22). During the early stage of type I diabetes, blood glucose levels are still normal (25). It is hard to detect the development of type I diabetes until massive destruction of β-cells in the pancreas has occurred and impaired insulin secretion has resulted, which makes it difficult to prevent type I diabetes (25). The management of type I diabetes involves continuous blood glucose monitoring and regular insulin injections (26). Patients with type I diabetes are usually dependent on insulin injection throughout their lives.

Compared to type I diabetes, type II diabetes has a much more complicated pathogenesis. Genetic components, obesity, particularly abdominal adiposity, pancreatic β-cell dysfunctions and oxidative stress are all risk factors that contribute to the onset of type II diabetes (27). Type II diabetes represents 90% to 95% of all types of diabetes (19). It is characterized by both insulin resistance and a defect in insulin secretion. The degree of insulin resistance and insulin deficiency varies depending on the stage of development. During the early stage of type II diabetes, insulin resistance starts to occur while insulin secretion increases to compensate for the resistance. In this early stage of type II diabetes patients usually have a

normal blood glucose but are hyperinsulinemic (28). Later, insulin secretion fails to compensate for the insulin resistance, and the blood glucose level starts to increase to an abnormally high concentration. If hyperglycemia is not well controlled in a long-term, eventually the mass of pancreatic β-cells will shrink due to dysfunction (28, 29). Before the dramatic loss of β-cells in the last stage, the progression of diabetes can be reversed, and remission is found in type II diabetes patients with effective treatments (28). Even though type II diabetes is also believed to be associated with genetic factors, many previous studies have shown that type II diabetes can be delayed or even prevented in some high-risk population (30). Intensive lifestyle interventions, some specific drugs, and surgeries have all been shown to reverse the progression of type II diabetes (30-32).

b. Prevalence of type Ⅱ diabetes

According to the newest national report released in 2014, 29.1 million people in the USA had diabetes in 2012, which is 9.3% of the total population. Of those 29.1 million, 8.1 million people were undiagnosed (1). Based on the proportions of type I and type II diabetes, about 27 million people have type II diabetes, which is about 8.6% of the total population. Based on previous surveys, the average age of type II diabetes patients has shifted to a younger age (33). The prevalence of type 2 diabetes in youth (15-24 years old) has increased from 0.34 per 1000 in 2001 to 0.46 per 1000 in

2009 (33).

The economic costs of diabetes increased 41% in five years, from $174 billion in 2007 to $245 billion in 2012 (34). Of the estimated $245 billion, about $176 billion was for medical expenditures in hospitals, medications, and diabetic supplies for diabetes patients, and $69 billion was indirect costs of reduced productivity and mortality costs (34). The average cost for a diagnosed diabetes patient is $13,700 per year for medical expenditure (34). Undoubtedly, diabetes has brought great economic hardship for both governmental healthcare services and for the families of those with diabetes.

c. Metabolic characteristics of type Ⅱ diabetes

Hyperglycemia is the main characteristics of type II diabetes. Patients with type II diabetes have both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (35). IFG is characterized by high fasting blood glucose levels and IGT is characterized by high postprandial blood glucose levels. Patients with only IFG show more hepatic insulin resistance while patients with only IGT show more severe skeletal insulin resistance (36). Comparing both tests, glucose tolerance test showed higher sensitivity than fasting glucose tests in predicting insulin resistance (37).

Besides high blood glucose levels, many other metabolic abnormalities occur in type II diabetes. Type II diabetes patients commonly also have dyslipidemia with high blood LDL-cholesterol, high triacylglycerol (TAGs),

high free fatty acids (FFA) and low HDL lipoprotein levels (1). These abnormal concentrations of lipids and lipoproteins are thought to be caused by hepatic overproduction of VLDL (38), a triacylglycerol-rich lipoprotein. VLDL in circulation interacts with lipoprotein lipase, which hydrolyzes lipoprotein triacylglycerols to free fatty acids, producing VLDL remnants. Some of the VLDL remnants are converted to LDL (39). The effects of insulin include 1) enhancing FFAs or TAGs uptake by adipose and muscle tissues, 2) increasing non-fat substrates conversion to fat in adipose and liver tissue (this process is called *de novo* lipogenesis), and 3) suppressing lipolysis in adipose tissue (40). In addition, insulin has suppressive effects on VLDL apolipoprotein B (Apo B) production which is responsible for carrying lipids through the body (41). In insulin resistant syndrome (IRS) patients, the serum FFAs and TAGs are not efficiently taken up by muscle or adipose tissues. Instead, adipocytes have increased lipolysis rate to release more FFAs in serum and the elevated flux of FFA to the liver to increases substrate for hepatic VLDL synthesis. Moreover, in IRS patients, Apo B production is not suppressed. With increased concentration of all substrates, VLDL is overproduced in the liver, increasing release into blood to further increase serum TAG, FFAs and LDL cholesterol concentration (38, 42). Typically, in a non-insulin-resistant subject, after consuming a meal, insulin is released to suppress gluconeogenesis and promote *de novo* lipogenesis. However, in an insulin-resistant subject, gluconeogenesis is not suppressed by insulin while

insulin effects on promoting *de novo* lipogenesis is preserved (42). This "selective insulin resistance" mechanism causes coexistence of both hyperglycemia and dyslipidemia in insulin-resistance subjects (43).

Besides dyslipidemia, hypertension is also very common in type II diabetes patients. However, it is difficult to test whether insulin resistance or hypertension is the cause while the other is the consequence (44). The coexistence of insulin resistance and hypertension in many diabetic patients suggests that their pathological conditions may develop via a shared pathway, even though the mechanism is not fully understood (45). In 1980, Sleder et al. discovered that feeding rats a high-fructose diet for one to four weeks would induce hyperinsulinemia and insulin resistance in rats (46). Another study in 1987 by Hwang et al. suggested that the fructose-induce insulin resistant rat also developed essential hypertension (47). Many subsequent studies of fructose-fed animals confirmed the coexistence of insulin resistance and essential hypertension in this animal model (48). It has been suggested that prolonged fructose-feeding will increased salt absorption in the small intestine and renal proximal tubule in animal models and overtimes causes a salt overload that eventually leads to essential hypertension (49). Besides abnormal salt reabsorption, Lembo et al. observed in humans a sympathetic overactivity with increased norepinephrine release, caused by elevated insulin levels in essential hypertension patients, indicating that both insulin resistant and hypertension

patients have sympathetic nervous system overactivity (50). Moreover, Insulin has important role in stimulating the production of potent vasodilator, nitric oxide, from vascular endothelium. Patient with insulin resistance will has imbalance between the vasodilator and vasoconstrictor, which causes endothelial dysfunction and increase the risk of hypertension (51). However, this mechanism is poorly understood (52, 53).

d. Complications of diabetes

In 2010, diabetes was the 7th leading cause of death (1). Diabetes patients not only suffer from hyperglycemia, but experience a number of life-threatening complications. It has been suggested that the diabetic death rate might be underestimated due to complications caused by diabetes not being fully considered (54). One of the biggest issues that diabetes nurse management now emphasizes is the prevention and amelioration of diabetic complications (55).

1. Neuropathy, nephropathy, and retinopathy

Painful diabetic neuropathy (PDN) is one of the most common complications of diabetes, and affects about 21.5% of type 2 diabetes patients according to a community study conducted in the UK (56). The early symptoms always start in the lower limbs (hands, toes), with stinging, burning, or numbness feelings (57). The pathogenesis of PDN is still not fully understood (58). However, hyperglycemia is thought to be the major risk for

PDN, as elevated blood glucose levels accelerate protein glycation and oxidative stress, which may cause damage to the nervous system (59).

Another common complication of type II diabetes is nephropathy, which ultimately develops in 30 to 45 percent of type II diabetes patients (60). Hyperglycemia is believed to be the principle risk factor that causes nephropathy (61) and multiple mechanisms are involved in nephropathy induction.

With prolonged hyperglycemia in diabetic patients, glucose will bind to artery wall collagen or glomerular basement-membrane proteins and react non-enzymatically to generate advanced glycosylated end products (AGE) (62). AGEs, in Yamagishi's study, was confirmed to induce apoptosis and overexpression of vascular endothelial growth factor (VEGF) (63), which is a signal protein stimulates vasculogenesis, in mesangial cells. Mesangial cells are pericytes in blood vessels of the kidney. Glomeruli are clusters of capillaries that located in each nephron of kidney and the principle function of glomeruli is blood filtration and glomeruli are also considered to be the first site of urine production. With VEGF overexpression, mesangial cells of blood vessels in the glomeruli will be impaired with increased vascular permeability and eventually leads to hyperfiltration and proteinuria in kidney (64). Proteinuria is one of the remarkable characteristics of impaired renal function and diabetic nephropathy has two stages: microalbuminuria and macroalbuminuria. Microalbuminuria is defined as moderate albumin leakage

with 30 mg to 300 mg albumin in 24-hour urine collection. Macroalbuminuria is more than 300m albumin in 24-hour urine collection. The damaged renal system will increase sodium reabsorption as well as causing increased extracellular volumes, as a result, aggravate the high blood pressure (65). Therefore, insulin resistance and AGEs have played an important role in inducing hyperfiltration as a result of impaired renal function in diabetic patients.

Moreover, with high levels of blood glucose, the mRNA of GLUT-1 protein, which is the most common glucose transporter in renal cells, is overexpressed (66). To promotes the expression of GLUT-1, a cytokine named transforming growth factor beta (TGF-β), is produced by mesangial cells (67). TGF-β will increase the production of extracellular matrix and inhibit the extracellular matrix removal which causes the mesangial expansion (68), and mesangial expansion leads to the thickening of the glomerular basement membrane, which is the barrier that separates the vasculature from urinary space (69). Mesangial expansion changes the kidney structure, as a result, causes hyperfiltration, and increases the glomerular filtration rate (GFR), which is a test to predict renal functioning. Therefore, GLUT-1 overexpression and TGF-β induction have similar effect as AGEs, and scientists believe that mesangial expansion is the hallmarks of compromised kidney function (70).

Diabetic nephropathy is the most common syndrome that causes renal

replacement therapy (71) which brought many diabetic patients with physical suffering and economic hardship.

Diabetic retinopathy is a microvascular complication that affects almost all type I diabetic patients and about 60% of type II diabetic patients. Diabetic retinopathy has been the leading cause of blindness among adults in the US in the most recent two decades (72). Diabetic retinopathy is caused by damage to vessels in the retina, but it is difficult to detect in its early stage, until bleeding occurs and patients have blurred vision (73). Diabetic retinopathy is mainly caused by hyperglycemia, which induces mitochondrial overproduction of reactive oxygen species (ROS), resulting in vascular endothelial cell damage (74). Consequently, the retinal capillary basement thickens and diabetic macular edema develops. Strict glycemic control and blood pressure monitoring are very important to delay the onset and progression of diabetic retinopathy (72).

2. Increased risk for CVD

Cardiovascular diseases (CVD) and type II diabetes shared many risk factors and even pathogenesis (75). Hyperinsulinemia, hyperglycemia, obesity, dyslipidemia (high plasma TAG and low HDL cholesterol concentration) and elevated blood pressure, which are all common characteristics of type II diabetes, are also classified as risk factors for developing CVD (75). Thus, diabetes is not only a major risk factor but also a significant cause of CVD (76). Not surprisingly, CVD and strokes are the

major cause of death and disability among diabetic patients (77). Many factors have been shown to be linked with both diabetes and CVD, and oxidative stress is considered to be one of the principle contributors to the induction of diabetic vascular complications (78). Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) production and body's ability to detoxify the reactive species through antioxidants (79). Hyperglycemia in diabetic subject causes a mild inflammation, which induces an overproduction of ROS in the mitochondria. In many in vitro studies, increased levels of oxidized DNA (80), lipids (81) and proteins (82) has been observed with hyperglycemia (83). The generation of oxidative stress are believed to trigger the inflammatory response by activating the transcription factor NF-κB, even though the exact mechanism is still unclear (78, 84). NF-κB controls the transcription of many genes that affect immune responses (85). In general, NF-κB has protective effects to regulate cardiac myocyte apoptosis during the acute heart injury (86). However, prolonged activation of NF-κB appears to induce signaling pathways that generate the cytokines tumor necrosis factor α and interleukin-1. These two cytokines synergistically decrease myocardial function by depressing the production of myocardial nitric oxide (NO) (87) which is served as a cardiovascular signaling molecule that regulate cardiac physiology (88). In summary, long-term expose to hyperglycemia and prolonged activation of NF-κB can be a trigger to decrease myocardial function, induces endoplasmic reticulum stress

responses, and cell death in cardiac myocytes (89).

Besides increasing oxidative stress and inflammatory responses, elevated blood glucose levels typical of type II diabetes will also increase collagen glycation, which facilitates attachment of LDL particles to the collagen, and increases LDL retention on arterial walls, thereby inducing atherosclerosis The activation of NF-κB as mentioned above will also elevate the level of the growth factor vascular endothelial growth factor (VEGF). In atherosclerotic patient, macrophages take up oxidized LDL initiating the foam cell formation in atherosclerotic lesions and a prolonged high concentration of VEGF in circulation increases vascular permeability, as a result increasing the uptake of LDL by macrophages, which aggravate atherosclerosis (90, 91). In addition, as mentioned above, hypertension is also one of the major metabolic characteristics of type II diabetes because of the impaired endothelial function caused by insulin resistance, which also contributes to increased CVD risk (77). Moreover, the overproduction of VLDL from liver, increases VLDL remnants, dense LDL in circulation, which will raise the risk of atherosclerosis (77, 92). Overall, the overactive inflammatory responses in cardiac system, disordered blood pressure, aggravated atherosclerotic lesions and hyperlipidemia conditions all contribute to the elevated risk for diabetic cardiovascular disease. The death rate from CVD in type 2 diabetes patients over 18 years old is 1.7 times than non-diabetic CVD population according to the most recent national diabetes statistics report (1).

3. Non-alcoholic fatty liver disease (NAFLD) development

Non-alcoholic fatty liver disease (NAFLD) is a disease of abnormal hepatic fat accumulation exceeding 5–10% of liver weight. NAFLD is a spectrum of liver diseases progressing from steatohepatitis, to fibrosis, and finally to cirrhosis (93). Steatohepatitis is the first stage of NAFLD with lobular inflammation caused by fat accumulation which leads to liver enlargement (94). Liver fibrosis is a scarring process which represents the body's response to liver injury, where the extracellular matrix is overproduced and with excessive connective tissue builds up in the liver in this stage (95). Cirrhosis is the last stage of NAFLD, when scar tissue has replaced the healthy liver tissue, which blocks blood flow within the liver and inhibits hepatic tissue regrowth. Cirrhosis is considered irreversible and will progress to liver failure (96). Metabolic syndrome is highly predictive of the development of NAFLD (97). The prevalence of NAFLD in the general population is estimated at 20-30% (98), whereas in type II diabetes patients, the incidence may be as high as 87% (99). NAFLD was first associated with obesity in early 1990s (100, 101). More recently, it has been shown that hepatic and adipose insulin resistance are better predictors of liver dysfunction more than the body mass index (102, 103).

The exact mechanism of NAFLD development in metabolic syndrome patients is not fully understood (97), but patients with insulin resistance show a higher rate of lipolysis and a lower rate of lipogenesis within adipocytes,

and subsequently a greater concentration of plasma free fatty acids (FFAs) and triacylglycerol (TAGs), which increases the flux of FFAs to the liver (104). Moreover, the combination of high plasma glucose and insulin levels leads to greater hepatic de novo lipogenesis, which contributes to further accumulation of lipids in the liver (105). As discussed above, de novo lipogenesis is upregulated by insulin in non-diabetic subjects. However, unlike insulin signaling to glucose homeostasis, insulin signaling to induce de novo lipogenesis was preserved in insulin resistant patient (42, 106). It has shown in insulin-resistant patients that elevated serum insulin and glucose levels can stimulate synthesis of the transcription factor SREBP-1c, which promotes transcription of acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FAS) (106). ACC and FAS are both rate-determining enzymes in hepatic fatty acids synthesis, which elevates hepatic fatty acids levels, as excessive FFAs are esterified to TAGs and stored in liver (107).

Besides increased hepatic TAGs accumulation, impaired mitochondrial function is another factor which has been identified as a contributor to imbalanced hepatic lipid disposal (107). Hepatocytes are very rich in mitochondria, due to the high energy demands in this tissue (108). Mitochondria are responsible for fatty acid beta-oxidation and oxidative phosphorylation, which generate ATP. In an animal study, comparing healthy, normal rats to transgenic insulin resistant models, the insulin-resistant rats had swollen mitochondrial with hypodense matrix and broken

cristae (109). This mitochondrial dysfunction results in decreased respiration rate, ATP depletion and increased formation of ROS, which accelerate the apoptosis of hepatocytes (110) . This evidence suggests that impaired mitochondria function results in decreased fatty acids oxidation in patients with NAFLD, although the mechanism is not fully understood (111). In summary, the accelerated flux of external lipids, increased endogenous hepatic lipogenesis, and reduction in hepatic lipid oxidation from mitochondrial dysfunction are three main contributors that cause the accumulation of fat in the liver and the development of NAFLD. All these three factors are related to insulin resistance, which is consistent with the concept that impaired glucose tolerance may promote or accelerate the NAFLD progression.

In conclusion, diabetic patients can develop a number of debilitating complications. However, these complications can be minimized or prevented with good glycemic control and, perhaps, certain dietary interventions. Preventing and controlling these complications is of great importance in decreasing the morbidity and mortality of diabetes.

e. Dietary recommendation for type Ⅱ diabetes

The latest nutritional recommendations for diabetes released by the American Diabetes Association (ADA) is aimed at preventing or at least slowing down the progression of diabetes (2), and maintaining normal blood

glucose, lipids, and blood pressure levels (2). Moderate weight loss is recommended for overweight or obese type II diabetes patients in order to reduce hyperglycemia and CVD risk. Therefore, a negative energy balance should be achieved in order to lose 5-10% of body weight (112). Type II diabetic patients are also recommended to limit foods high in sugar, fat, carbohydrates, and alcohol, and consume a variety of fruits and vegetables (113). A high dietary fiber intake is thought to increase insulin sensitivity and pancreatic functioning, therefore fiber consumption may improve hyperglycemia in type II diabetes patients (114). Type I and II diabetic patients are recommended to consume at least the U.S. Department of Agriculture (USDA) recommendation for dietary fiber for the general population, which is 14 g fiber/1,000 kcal (2). Some studies show that a very high fiber diet (50 g fiber/ day), and particularly a high intake of soluble dietary fibers, may further help in improving hyperglycemia, hyperinsulinemia and hyperlipidemia (115). Also, the nutritional recommendations encourage patients with diabetes to consume low-glycemic index foods that are high in fiber and other important nutrients (2). The concept of glycemic index was first developed in 1981, when it was found that among foods with the same carbohydrate content, the glucose and insulin response may vary significantly (116). The glycemic index was defined as the incremental area of the blood glucose response after consuming a fixed amount of carbohydrate from a food compared to the same amount of carbohydrate

from white bread. The result is expressed as the percent of the area compared to the control area (white bread) (117). Low glycemic foods are defined as 55% percent or lower incremental area of the control (118). Some examples of low glycemic foods include beans, oats, barley and lentils (119). It has been found that over 6-12 weeks of low-glycemic food intake, subjects showed a small but clinically significant reduction in the percentage of glycated hemoglobin (%HA1c) compared to subjects consuming high-glycemic foods (119). %HA1c is an index of long-term blood glucose concentration. It has also been found that high-fiber, low glycemic foods can improve postprandial glucose and insulin responses in diabetic subjects (120).

Overall, dietary recommendations for type II diabetic patients are aimed at slowing the progression of diabetic complications by improving glycemic control and by decreasing energy intake to archive moderate weight loss. Consuming a low-glycemic, high fiber containing diet is an important approach to controlling postprandial glucose levels.

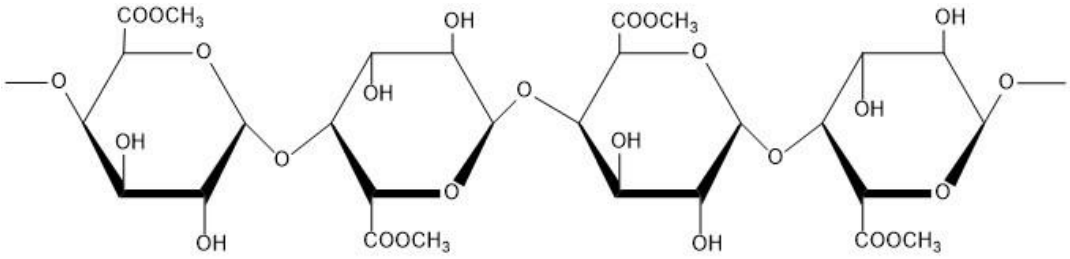
II. Dietary fibers

a. Definition of dietary fiber

According to the Panel on the Definition of Dietary Fiber, dietary fibers are defined as non-starchy polysaccharides and lignin that are non-digestible or poorly digested by humans because of the lack of specific enzymes in the

mammalian intestinal tract (121). Dietary fibers were formerly classified into two categories, soluble and insoluble, a chemical classification rather than a physiological one (122). Based on their physiological properties, soluble fibers are usually highly fermented and viscous, whereas insoluble fibers are generally only marginally fermented and have little or no viscosity (122, 123). The distinction between soluble and insoluble fiber is more related to the chemical structure or the food processing method than the molecular composition of the fiber (122). To address the physiological response of fibers intakes, it is necessary to measure the viscosity and fermentability of a fiber within the intestinal contents of the subjects (124). Some examples of water soluble and well fermentable fibers are pectin, guar gum, beta-glucans, and arabinoxylan, while some examples of water insoluble and less fermentable fibers are psyllium, cellulose and chitin. The soluble fibers typically have polar, hydrophilic groups (hydroxyl, carboxylic acid etc. functional groups) which can interact with water molecules. For example, pectin has a linear structure with hydroxyl group available to be exposed to water (Figure 1). Even though arabinoxylan does not have a linear structure, its hydroxyl functional groups are still exposed to the solution which makes it soluble (Figure 2). On the contrary, cellulose is a linear structure with hydroxyl group (Figure 3), however, its hydroxyl groups interact with each other which make the chains twist together like a rope and the hydroxyl groups are not available to interact with water molecules which makes it

insoluble (shown in Figure 4). However, some fibers, like beta-glucans and cellulose, have different physiological properties even though they are composed of the same sugar, namely glucose. The viscosity and fermentability of beta-glucan can vary depending on the source, molecular weight, molecular structure, and degree of branching (125). Cereal beta-glucan from oat, barley, wheat or rye are found in the cell walls of the endosperm, and has a (1,3)-β-linked and (1,4)-β-linked linear backbone (Figure 5) with high viscosity and fermentability (117). The beta-



Pectin

Figure 1: Structure of pectin

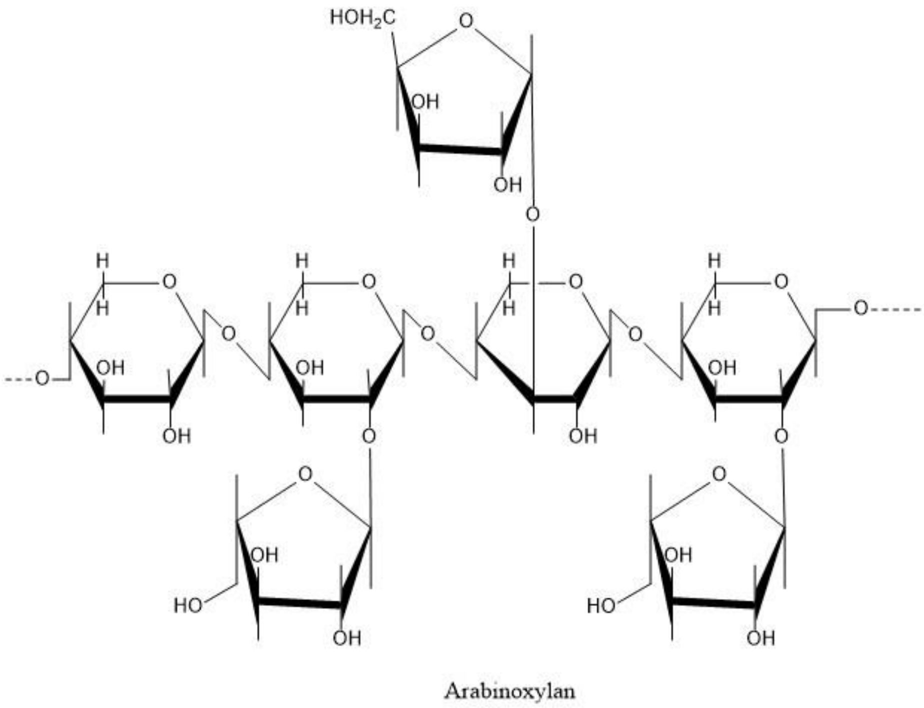


Figure 2: Structure of arabinoxylan

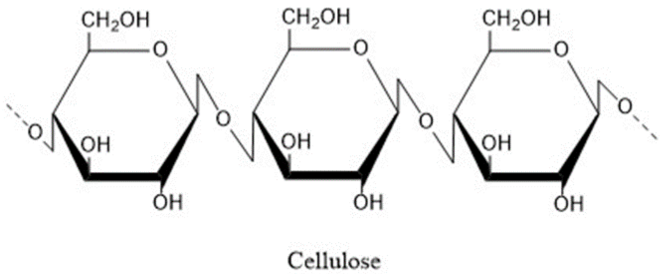


Figure 3: Structure of cellulose

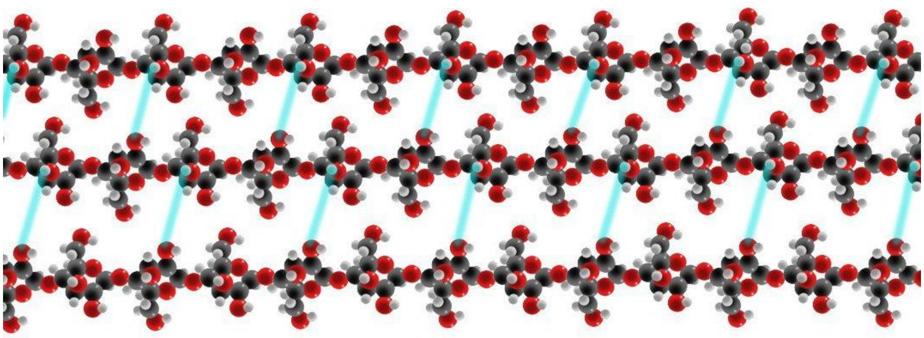
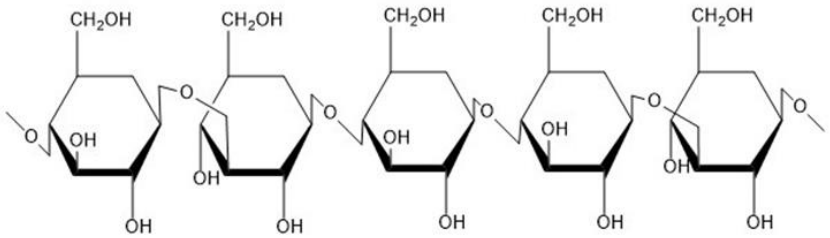
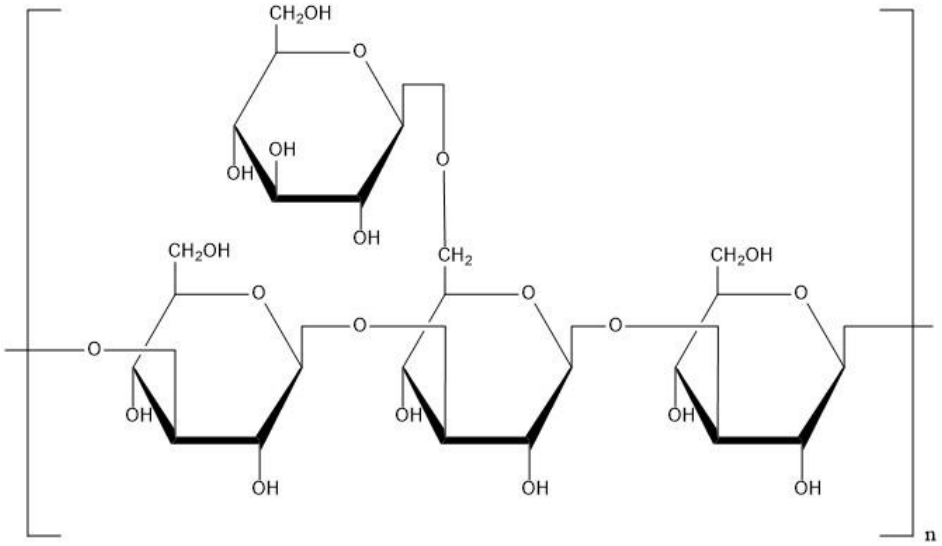


Figure 4: 3-D structure of cellulose



Cereal-derived Beta-glucan

Figure 5: Structure of cereal-derived beta-glucan



Yeast-derived Beta-glucan

Figure 6: Structure of yeast-derived beta-glucan

glucan from yeast or mushrooms are located in the cell walls, and consist of (1,3)-β-linked backbones with (1,6)-β-linked side chains (Figure 6). These beta-glucans have low viscosity with less fermentability (118). Waszkiewicz-Robak proposed that the yeast beta-glucan has a higher degree of polymerization (DP) with long, multi-branched side chains, which contribute

to its insolubility (125). Besides these natural fibers, some dietary fibers are semisynthetic, that they are derived from natural sources, but chemically modified. Hydroxypropyl methylcellulose (HPMC) is one of these semisynthetic dietary fibers, as it is derived from cellulose. It is nonfermentable but highly viscous and can be added to other food products or solution as a viscosity modifier (119, 120). Even though HPMC is derived from cellulose, it does not have the “rope structure” like cellulose, with its hydroxyl functional groups, and so can be dissolved in water (Figure 7).

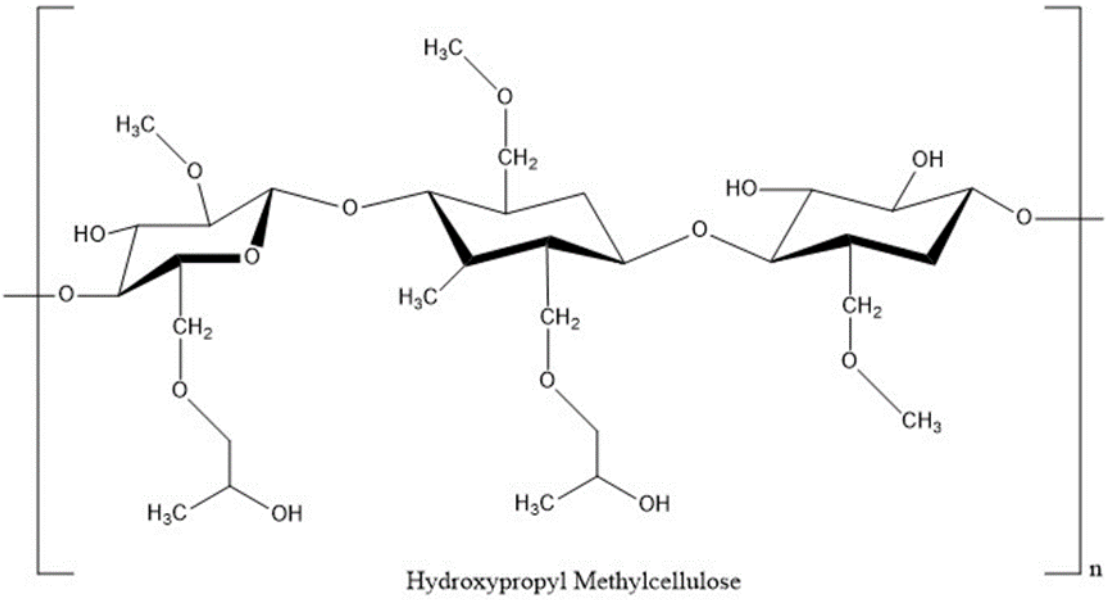


Figure 7: Structure of hydroxypropyl methylcellulose (HPMC)

It is worth mentioning the concept of prebiotics. Prebiotics are non-digestible, fermented food ingredients that can selectively stimulate the beneficial microbiota and potentially suppress the harmful bacteria dominant (126). The term prebiotics was first defined by Gibson in 1995, who updated this concept in 2005 with three criteria: 1) prebiotics are resistant to gastric acids

and will not be hydrolyzed by the enzymes in the mammalian gastrointestinal system. 2) prebiotics can be fermented by intestinal bacteria in the mammalian large intestine, and 3) prebiotics can selectively stimulate the growth or activity of intestinal microflora that promote gut health (127). All discovered prebiotics are fibers while not all the fibers qualify as prebiotics (128), which will be discussed later.

b. Physical and chemical characteristics

Viscosity

Viscosity was defined by Newton as the ratio between the flow of a fluid and the resistant force directed to the flow (123). When viscosity refers to fiber, it reflects the ability of a polysaccharides to thicken or form gels within fluids (123). Only soluble fibers can bypass digestion in the intestinal tract and produce viscosity in the intestinal contents (129). There are several factors that will affect the viscosity of the intestinal contents, including fiber concentration, molecular weight, processing method, temperature, pH of the fluid, and the time (123, 130). For example, different amounts of guar gum were added to the diets of growing pigs, and the viscosities of the digesta showed a positive, non-linear relationship with guar gum concentrations in their corresponding diets, which indicated that the viscosity of the intestinal contents has a positive relationship with the amount of soluble fiber consumption (131). In an in vitro study, with equal concentration, five fractions of the guar galactomannan with different molecular weights were

tested for viscosity under a viscometer (132). The results showed that the molecular weight of a fiber has a positive, non-linear relationship with its intrinsic viscosity (132). Besides molecular weight and fiber concentration, different processing methods were used to hydrolyze the same beta-glucan. It was found that aqueous extraction of beta-glucan samples had a lower viscosity compared to an alcohol-based enzymatic process for extracting beta-glucan samples (133). In addition, the pH of the solution is also considered to be a significant factor to influence the viscosity. It was found that the increase in hydrogen ion concentration (i.e. lowering of the pH) reduced the viscosity of guar gum solutions in the animals' intestinal tracts (134). In contrast, Cameron-Smith found that guar gum in acidified solution has higher viscosity compared to the guar gum in saline solution which is inconsistent with the previously described results. However, Cameron-Smith found that xanthan gum and methylcellulose showed no significant alteration in viscosity in acidified solutions compared to the viscosity in saline solutions(135). Therefore, how pH alters a fiber's viscosity may depend on the components of the fiber and other factors, and the exact mechanism is still unclear. After the animal models consume a type of soluble fiber, the viscosity in intestinal tract will change over time. In one study, the viscosities of different fibers were measured every 2-3 hours in a simulated intestinal tract environment, and the results suggested that viscosity will increase and reach a peak 2-9 hours after fiber consumption (130). However, with different

soluble fibers, the time and the levels of the peak may vary.

Fermentability

Fermentability is another very important characteristic of dietary fibers that has been related to health benefits. The main factors that affect the fermentability of a fiber are physiochemical characteristics (solubility, monomer structure, glycosidic bonds or the degree of branching), intestine transit time, and microflora composition (136). It has been shown that, generally, soluble fibers are more quickly fermented than insoluble fibers (137). For insoluble fibers, the total surface area of the fiber matrix available to digestive enzymes and the intestinal microflora has a positive linear relationship with the degree of fermentation. Therefore, insoluble fibers with high porosity and large particle sizes have greater fermentability (138). In addition, the complexity of the fiber structure has been related to its fermentability, as shown by Glitso et al. (139), different structures of arabinoxylan fibers were degraded to different degrees in the colons of pigs, and that arabinoxylans with more complex structure and more branching were less fermented .

Fermentation in the large intestine is carried out by anaerobic microbes, who metabolize dietary fiber to produce energy for microbial growth. A number of end products are generated through this process, including short-chain fatty acids (SCFA) and gases such as carbon dioxide, methane and hydrogen (140). The predominant SCFAs produced by colonic fermentation

are acetate, propionate and butyrate (141). The percentage of acetate, propionate and butyrate generated by fiber fermentation are relatively consistent, with a molar ratio of 60:20:20, regardless the fiber type (142). However, the levels of fiber degradation can be affected by colonic transit time and the composition of colonic microflora. Colonic transit time refers to the time needed for fiber containing foods to pass through the colon. Previous studies suggest that the mean transit time of a certain fiber has an inverse relationship with SCFA production, and that degree of fermentation is positively related to the fecal pH (143, 144). Besides the transit time, the endogenous gastrointestinal microbial flora in host influences fiber fermentation as well. The composition of the microbiome differs in each individual, and the composition is determined by host's genotype and physiology (145). Even within the same individual, the composition of microflora can vary from daily food intakes, age, and other factors (142, 146).

Particle size, surface area, bulk volume and hydration properties

Some other physio-chemical characteristics of dietary fiber that may affect the physiological effect of dietary fibers including the particle size of the food, surface area and bulk volume of fiber matrix, and fiber's swelling and water retention capacity (147). The particle size of a food depends on the type of the plant cell wall and the degree and type of processing (137). The water swelling capacity describes the amount of liquid a polysaccharide can absorb. As foods high in fiber dissolved in water, the fiber may swell and the

food particle size and the bulk volume of the fiber matrix may increase. Therefore, the wet form of the fiber might be a better model to mimic the scenario of digestion in intestinal tract (137). As discussed above, increased particle size, food bulk volume, and surface area will contribute to better accessibility of intestinal enzymes and microflora to its substrate, which enhances the fermentation process of the fiber matrix (137). The higher water binding capacity fibers can also increase fiber viscosity (148), increase stool output, and promote gut functioning (149).

c. Biological effects of viscous fibers

Cholesterol-lowering

Viscous fibers have been shown to reduce plasma cholesterol concentrations (8, 15, 150). In a human study, high viscosity fiber, HPMC, was found to be effective in reducing serum cholesterol in patients with primary hypercholesterolemia (151). Reductions in both total and LDL cholesterol are found (8, 152), whereas typically no change in triacylglycerol or HDL cholesterol are found (152). The mechanism of LDL reduction from viscous fiber intake is elusive; the speculation is that viscous fibers may function to impair bile acid reabsorption in the small intestine (153), although there is also evidence that viscous fiber reduces cholesterol absorption (154, 155). Levrat-Verny et al. proposed that feeding rats with guar gum and xanthan gum, which are both viscous fibers, can significant reduce the

plasma cholesterol concentrations and liver cholesterol contents compared to the control rats with no added viscous fibers (158). To investigate the mechanism of viscous fiber in lowering adiposity in rats, Levrat-Verny et al. measured the cholesterol metabolism in rats and discovered that the rats fed on viscous fibers (both guar gum and xanthan gum) had significant lower levels of cholesterol absorption with significant higher steroid excretion in fecal. The results suggested that the rats had viscous fibers intakes had significant lower total steroid balance and higher bile acids turnover rate than the rats without viscous fiber intakes, which contributes to lower serum cholesterol levels and lower hepatic cholesterol accumulation (158). In Lia's study, similar effects of significantly increased steroid excretion and decreased reabsorption of bile acids were observed in the rats fed with unprocessed oat bran fiber, which is high in viscous beta-glucan fiber, compared to the rats fed with β-glucanase processed, degraded oat bran fiber with no viscosity. This finding suggested that viscous fibers may obstructing the reabsorption of bile acids in the terminal ileum which elevate the steroid excretion and accelerate the bile acid turnover rate to decrease the cholesterol in the body (159). To compensate for the loss of bile acids in feces, LDL are taken up into the liver and the LDL cholesterol is used to synthesize bile acids, which lowers LDL cholesterol levels (156). Ironically, hepatic cholesterol synthesis increases (157), but not sufficiently to compensate for the reduction in cholesterol due to shunting into bile acid

synthesis.

As mentioned above, some viscous fibers can be fermented in large intestine and produce short chain fatty acids: acetate, propionate and butyrate. These SCFAs generated by fiber fermentation were believed to have hypocholesterolemia effects in many animal and human studies. Hara et al. conducted a study in 1999 when he fed the rats with SCFA mixture of acetate, propionate and butyrate, and observed a decreased hepatic and small intestine mucosal cholesterol synthesis rate compared to the rats fed with fiber-free diet (158). In 2015, Besten et al. found that the mice fed with acetate, propionate and butyrate salts suppressed peroxisome proliferator–activated receptor-γ (PPARγ) expression and activity (159). PPARγ is a type of nuclear receptor protein which controls fatty acid storage and glucose metabolism. The suppression of PPARγ promotes mitochondrial fatty acid oxidation as a result reducing lipogenesis and fat accumulation in hepatocytes and adipocytes.

In summary, viscous fibers obstruct cholesterol absorption and bile acid re-absorption. Hepatic uptake of LDL increases, and the cholesterol within LDL is used to synthesize the bile acids to replace those lost. Also, the SCFAs produced by viscous fiber fermentation can further reduce plasma cholesterol levels by inducing nuclear receptor protein, PPARγ, to switch fat metabolism from lipogenesis to fat oxidation.

Improvement of glycemic control

Viscous fibers intake has been shown to have favorable effects on glycemic control. For example, hydroxypropyl methylcellulose (HPMC), a viscous but non-fermentable dietary fiber, has been shown to reduce the postprandial glucose response in normal humans (160) and in non-insulin-dependent diabetic subjects (161). The effects of viscous fibers on the incremental plasma glucose area under the curve (AUC) may be up to a 25% reduction (162), although typically fasting plasma glucose concentrations are not affected (163). Panahi (133) used different hydrolyzing methods to process oat beta-glucan, a viscous fiber, and tested the postprandial glucose responses in healthy subjects after drinking the beverage containing these two different oat beta-glucan samples (133). The results suggested that with the same dietary fiber, increased viscosity can further decrease the postprandial glucose levels in healthy subjects, indicating that viscosity is one of the major factors related to postprandial glycemic control (133). The mechanism by which viscous fibers lower the postprandial glucose response include three possible pathways: First, the viscous fibers with high water swelling capacity can absorb the water to form a viscous gel or dispersion in the small intestine, which builds a mechanical barrier to slow glucose diffusion to the intestinal brush border to be absorbed (164). Second, viscous fiber has an effect of delaying gastric emptying, by an uncertain mechanism (165), which slows down macronutrient absorption from the gut and delays

and reduces the peak postprandial glycemic response. Jenkins et al. found a significant lower glycemic response to viscous fiber blended bread than to white bread in both healthy and diabetic volunteers (166). In another human study, type 2 diabetic individuals who consumed no viscous fiber in a beverage had a significantly faster gastric emptying rate, with a higher postprandial glucose level, compared to the individuals who consumed a beverage with viscous oat beta-glucan fiber, indicating that a delayed gastric emptying rate may contribute to the reduction of postprandial glycose response with viscous fiber intakes (167). Third, by delaying the gastric emptying rate, the viscous fibers can affect postprandial gastrointestinal (GI) hormone secretion in gastrointestinal tract, as a result, suppressing postprandial appetite, increasing satiety postprandially, and decreasing food intake (168). In a study by Juvonen et al. healthy individuals consumed beverages containing added modified oat beta-glucan, with and without viscosity. Individuals consuming the higher-viscosity beverage had a significant delayed gastric emptying rate compared to the individuals with the lower-viscosity beverage. In addition, those consuming the high viscosity beverage had decreased postprandial serum response of cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), which are all hunger-related hormones thought to stimulate appetite (168). Similarly, in healthy human subjects, the viscous fiber, solubilized cellulose, was added to test meals, and a decrease in postprandial plasma CCK was recorded, again

suggesting that viscous fiber increased satiety. (169).

Decreased adiposity

In an epidemiological study across 7 countries, dietary fiber intake and physical activity were found to be the only two factors that significantly inversely associated with subcutaneous fat thickness (170). Rats fed two different high viscosity fibers, HPMC and guar gum, in a background of a high fat diet, showed reduced adiposity with high viscous fibers compared to the low-viscous fiber group and no fiber added group, indicating that viscous fibers reduce adiposity in rodent (171). In another animal study, similar effects were observed in rodents. The rats feeding a high fat diet with HPMC had lower epididymal fat pad weights, adipose tissue gains and lower leptin concentration in circulation compared to the rats feeding the high fat diet with cellulose, which is a non-viscous dietary fiber (172). Leptin is a product of the obese gene (173), and it has been showed that people with obesity are typically with leptin resistant and high levels of leptin in circulation were observed with high fat mass (174). A significantly lower plasma leptin concentration was observed in rats with viscous, non-fermentable fiber intakes compared to the rats fed fermentable, non-viscous fibers, indicating that viscosity played an important role in reducing adiposity-related hormones (175). In addition, for those viscous and fermentable fiber, the SCFAs produced by fiber fermentation can induce nuclear receptor protein, PPARγ, to switch fat metabolism from lipogenesis to fat oxidation in adipose tissue as

a result decreasing adipose tissue gain and accumulation fat pad (159). Moreover, as discussed above, the viscous fiber intakes are related to decreased postprandial hormones response of CCK, GLP-1, and PYY, which suppresses the appetite and decrease the food intake (168). As a result, with less energy consumption, the subjects with viscous fibers intake tend to have higher weight loss and lower adiposity (176).

Decreased fatty liver

Nonalcoholic fatty liver disease (NAFLD), as mentioned above, is an abnormal accumulation of hepatic fat, which includes a series of liver conditions from steatohepatitis, fibrosis, to cirrhosis. NAFLD is a common co-incident disease with type II diabetes. The metabolic characteristics of NAFLD patients includes approximately 50% higher hepatic lipolysis rate and 30% higher hepatic gluconeogenesis rate (111). Dietary treatment is recommended for all stages of NAFLD (177), and increasing dietary fiber is highly recommended for NAFLD patients (177).

A number of animal studies support that viscous fibers can reduce hepatic lipid accumulation. For example, in high-sucrose-fed rats, those fed a fiber called PolyGlycopleX (PGX), a product containing three different viscous fibers, showed a lower hepatic steatosis compared to the animals without PGX feeding (178). In another study, rats fed high fat diets with either HPMC or guar gum both showed a lower liver lipid concentration compared with the rats fed control high-fat diet (171). In this study, hepatic gene

expression of G6Pase and PEPCK, two gluconeogenic enzymes (179), were lower in rats fed the viscous fibers (180). Viscous fiber intake may decrease hepatic gluconeogenesis by increasing hepatic insulin sensitivity. Since NAFLD is associated with decreased insulin sensitivity, this may explain the alleviation of NAFLD with viscous fiber intakes (181). This conclusion was also affirmed by another study in which HPMC was fed to rats as part of a high fat diet. Rat fed HPMC-containing diets had dramatically decreased gene expression of sterol regulatory element-binding protein-1c (SREBP-1c) and stearoyl-CoA desaturase-1 (SCD-1), two proteins that participate in lipid metabolism (182). SREBP-1c is a key transcription factor controlling de-novo-lipogenesis-related genes (183) while SCD-1 is an important enzyme producing unsaturated fatty acids (184). Decreased SREBP-1c and SCD-1 gene expression indicates decreased hepatic lipogenesis. Also, reduced plasma glucocorticoid has been observed in the rodents fed viscous fibers (182), which is significant, as glucocorticoid is a predisposing factor for whole body insulin resistance and fatty liver (185). The above studies indicate that viscous fibers may alter gene expression related to hepatic gluconeogenesis and lipid metabolism. As a result, hepatic insulin sensitivity will be improved, and fat accumulation in the liver will be reduced by lower de novo lipogenesis, increased fatty acids oxidation, and triglycerides hydrolysis.

Summary of health benefits of viscous fibers

In conclusion, viscous fibers can increase bile acids excretion, due to

blocking of bile acid re-absorption, and as a result lower plasma cholesterol concentration by increasing hepatic uptake of LDL. It will also reduce the rate of absorption of glucose in the intestine, thereby lowering the postprandial glucose response. Viscous fibers also appear to decrease adiposity. Thus, consumption of viscous fibers should improve the lipid profile, glycemic control, body composition, and fatty liver condition, particularly in diabetic patients.

d. Biological effects of fermentable fibers

Even though most soluble fibers are both viscous and fermentable (122), evidence suggests that viscosity and fermentability play different roles in modulating metabolism (186).

Prebiotic dietary fibers

The updated criteria for prebiotics as mentioned before includes 1) resistance to digestion in the mammalian gastrointestinal system, 2) fermentation by intestinal microflora in subject's colon, and 3) selective stimulation of the growth or activity of intestinal microflora that promote gut health (127). Commonly accepted beneficial microbiota include *Bifidobacteria* and *Lactobacilli*. The fermentable fibers which promote the growth and activity of *Bifidobacteria* or *Lactobacilli* are considered to qualify the third criteria of prebiotics. Roberfroid in 2007 proposed that based on this criteria, only two dietary fibers are considered as prebiotics which are trans-

galactooligosaccharide (TOS) and inulin (187). Some other studies suggested that pectin, barley β-glucan, resistant starch, galactooligosaccharides (GOS), fructo-oligosaccharides (FOS) and xylooligosaccharides (XOS) are also qualified as prebiotics (128, 188-191). In many studies, very low consumption of inulin, FOS or GOS ingestion (15-40g/day) will result in the increased counts of *Bifidobacteria* in the colon but unchanged total fecal bacteria (128, 188, 192, 193). Even though many viscous fibers are considered to have effects on controlling postprandial glycemic response and decreasing adiposity as we discussed above, the health benefits of prebiotics dietary fibers are considered to be substantially more limited, mostly towards gut health (128). A number of health benefits of prebiotics have been proposed, including 1) attenuating symptoms and inflammation of infectious gastroenteritis, 2) preventing colon cancer, 3) promoting weight loss and reducing obesity, 4) reducing CVD risks, 5) improving mineral bioavailability of calcium, 6) shortening the duration and relieving the conditions of infectious diarrhea (194).

As prebiotic dietary fiber can be fermented in large intestine, the end products of fermentation, the SCFAs butyrate, acetate, and propionate, are considered to benefit the gut in many different ways (195). SCFAs production in the intestinal tract will lower the colonic pH, which limits growth of pathogens (196). A low pH in the large intestine is also improves bioavailability of calcium. Enhanced calcium absorption from the lower

colonic pH likely occurs due to more ionic calcium, which accelerates passive calcium diffusion (197, 198). Acetate, propionate, and butyrate generated by fiber fermentation in the colon are absorbed by enterocytes and then transported in the portal circulation into the liver, then eventually the peripheral venous blood system (141). There are two mechanisms for SCFAs absorption: One is that protonated SCFA cross through the mucosal cytosol by positive diffusion involving uptake of luminal HCO_3 (199); Another approach is non-ionized SCFAs are taken up through active sodium and chloride coupled absorption (200, 201). Most butyrate is utilized in the intestinal mucosa for energy (202). The acetate and propionate are transported in the portal circulation (203) where propionate is taken up by the liver, where it can be used as a primary precursor for gluconeogenesis (204). Propionate is also proposed to have suppressive effects on hepatic lipogenesis (205). It has been shown that with high levels of propionate perfusion, hepatic cholesterol and fatty acid biosynthesis in rats were significantly decreased compared to the rats with no propionate perfusion (206, 207). However, the suppressive effect of propionate on hepatic lipogenesis was not observed in humans, as it was in rats, and consequently the cholesterol-lowering effect of propionate remains controversial (208). Acetate, as the major end product from fermentation, is the main SCFA in circulation and it can be taken up by adipose, hepatic and muscle tissue as a primary substrate for lipogenesis and cholesterol synthesis (204). However,

acetate is also proposed to enhance fatty acids oxidation by upregulating the expression of peroxisome-proliferator-activated receptor α (PPARα), which is the nuclear receptor protein that regulates mRNA expression of fat oxidation enzymes (209). Kondo et al. fed mice a high dose of acetic acid and observed significantly elevated genes expression of PPARα and significant reduction in serum cholesterol and hepatic lipid contents, compared to control mice not given acetic acid, indicating the suppressive effect of acetate in body fat and liver lipids accumulation (209). Butyrate is thought to be particularly useful in enhancing gut health by its anti-carcinogen effects on controlling intestinal cellular differentiation and proliferation (210). An in vitro study by Ruemmele et al. in 2003 found that butyrate can induce the apoptosis via mitochondrial pathways in colon cancer cells (211). Also, butyrate is a preferred fuel source for normal gut epithelium more than acetate and propionate (202), and butyrate promotes colonic normal cells proliferation in intestinal mucosa during the growth or repair after injury (212). In vitro, deprivation of butyrate induced massive apoptosis and causes overloaded macrophages in intact colonic mucosa (213). Therefore, butyrate can selectively stimulate growth of the normal colonic cells while inducing death in colonic carcinoma cells (214).

Fermentable, non-prebiotic dietary fibers

Some fermentable fibers lack of evidence promoting the growth or activity of *Bifidobacteria* or *Lactobacilli*, and therefore fail to qualify as a

prebiotic. However, they can still provide health benefits via production of SCFAs as discussed above. The SCFAs produced by fiber fermentation can decrease lipogenesis and increase fat oxidation in liver and adipose tissue as a result decreasing cholesterol synthesis and accumulation in liver and fat pad, killing pathogens and improve mineral bioavailability.

e. The effect of yeast derived beta-glucans

Yeast derived beta-glucan, as discussed in the fiber structure section above, is a type of insoluble fiber with low viscosity and some fermentability (215). Even though the effects of beta-glucan in glycemic control (16, 133, 216-219), improving lipid profiles (8, 15, 157) and reducing NAFLD progression(18, 217) has been shown in many studies, most of the studies used cereal derived beta-glucan, which may have different physiological characteristics than yeast derived beta-glucan. As we discussed above, yeast beta-glucan is not soluble, and has less viscosity and fermentability than cereal beta-glucan. There are a few papers proposed that yeast beta-glucan can reduce blood glucose in rodents (220-222), however, Angela et al. proposed that whole body insulin sensitivity is unchanged with yeast beta-glucan oral consumption (223). Angela et al. also found that yeast beta-glucan can enhance adipose mRNA expression and circulating levels of anti-inflammatory immune regulatory cytokine IL-10, which may contribute to the health benefits to the yeast beta-glucan consumption, although the

mechanism remains unclear (223). The effects of yeast beta-glucan are also related to immune system stimulation and reducing lipid profiles. Mice fed yeast beta-glucan showed a dose-dependent reduction in plasma cholesterol levels (224), and significantly increased the concentration of peripherals monocyte and macrophages in the mice, indicating an improved immune response (224). Similarly, obese human subjects fed 15 g yeast beta-glucan per day for eight weeks had significantly reduced total and LDL cholesterol levels compared to their baseline, while no significant difference was observed for triglyceride concentrations (225). Yeast derived beta-glucan was also found to be recognized by one of the pattern recognition receptors (PRRs), dectin-1, to induce innate immune response in rodents (226). PRRs are proteins produced by innate immune system cells to identify pathogens or cellular damages. Yeast beta-glucan injection to the pre-diabetic mice can induce antigen–specific autoimmune response in pancreatic beta cells by enhancing the expression of some anti-inflammatory immune regulatory cytokines (IL-2, IL-10 and TGF-β) and modulating T cells responses against pancreatic beta cell antigens, as a result delaying the onset of hyperglycemia and protect the rodent from type I diabetes (226). In another study in overweight or obese humans, feeding yeast beta-glucan for 4 weeks significantly elevated both plasma levels and the adipocyte mRNA expression of cytokine IL-10 (223) which also confirmed the promoted autoimmune response by yeast beta-glucan. Increasing mRNA expression of

IL-2 can reduce serum lipids concentration, which was first discovered by Wilson et al. in 1989 (227), and was confirmed by following in human studies (228). Also, Querfuld et al. found that IL-2 can generate a dose-dependent suppressive effect on lipoprotein lipase (LPL) production in vitro in human macrophages (229). Decreased LPL can inhibit the triglycerides hydrolyzing in lipoproteins, as a result lower the cholesterol in circulation. Besides IL-2, TGF-β activation by yeast beta-glucan, also showed to up-regulate bile acids synthesis which promoted cholesterol clearance in circulation (230). Moreover, yeast beta-glucan was showed to be effective in attenuating oxidative stress in both animal and human studies, yeast beta-glucan was found to significantly lower malondialdehyde (MDA) concentration in circulation (231, 232) and MDA is a product from lipid peroxidation which represents the free radical productions, and lipid peroxidation levels are closely correlated to lipid metabolism and lipid profiles (233). It has shown that increased circulating MDA can significantly improve lipid profile by lowering HDL and decreasing total cholesterol, LDL and triglycerides in both healthy and type II diabetic human subjects (233).

III. Animal models of diabetes

To investigate diabetes, animal models are widely used to mimic the disease in human. There are two different types of animal models used to study diabetes - genetic models and chemically induced models.

a. Genetic models

The most common genetic diabetic models are the ob/ob mouse and Zucker Diabetic Fatty (ZDF) rats.

The Zucker fatty rats were first found in 1961 by Lois Zucker, who discovered two fat rats that had normal weight brothers, which indicated the increased weight developed from a genetic variation (234). Zucker defined the gene she found as the “fa” gene, which was found to be recessive (234, 235). Only the fa/fa rats are obese, while the FA/FA or FA/fa have normal body weight. The obesity of fa/fa rats comes from a leptin receptor mutation, which results in a loss of food intake control (236). Rats deficient in the leptin receptor will not feel satiety, which leads to overeating (237). Long-term feeding (>5weeks) will result in overweight, hyperglycemia, hyperinsulinism, insulin resistance, hyperlipidemia and mild hypertension in Zucker fatty rats (237). A substrain of the Zucker fatty rat is the Zucker diabetic fatty (ZDF) rat, which are more insulin resistant but less obese (238). Studies show that ZDF rats have a higher pancreatic beta cell apoptosis rate, which then limits their ability to secrete insulin when hyperglycemia develops (239). ZDF rats are used as a model to investigate type II diabetes exhibiting both insulin resistance and beta cell dysfunction (237).

Ob/ob mice were first discovered in 1949 at the Jackson Memorial Laboratory (240). The ob/ob mice have a genetic mutation in the leptin gene, and thus do not secrete leptin (241), resulting in hyperphagia and obesity

(242). They eventually develop insulin resistance, hyperglycemia, and hyperinsulinism, similar to type II diabetics (243). Although the ob/ob mouse is an often used model of type II diabetes, leptin deficiency in humans is extremely rare (244), suggesting that ob/ob mice may not be a good model for human diabetes (245). However, ob/ob mice are found to be very useful to study functioning of pancreatic beta cells in diabetes (245).

Both ob/ob mice and ZDF rats are used as models for obesity-induced diabetes, which is type II diabetes (237). However, whether these genetic models of obesity with diabetes are a good model of human type II diabetic patients, which is usually due to obesity, is still questionable (237). Compared with ob/ob mice, ZDF rats seem more similar to obesity-induced diabetes with impaired pancreatic beta cell functions (237).

b. Chemically induced diabetes

Methods to chemically induce diabetes in rodents are still common, partly due to the high cost of genetic models of diabetes, and their uncertain relevance to human diabetes. A frequently used chemical to induce diabetes is streptozotocin (STZ), which was first identified in 1960 as an antibiotic produced by a soil microbe (246). In 1967, it was found to have diabetogenic effects in dogs and rats by massively damaging pancreatic beta cells, leading to reductions in plasma insulin level to 5% or less of normal within 24 hours of injection (247). STZ is believed to induce intracellular gene damage by

DNA alkylation in β cells (248). In 1983, Sandler et al. proposed that, after STZ injection induced DNA alkylation, poly-ADP ribose polymerase is activated to synthesize and repair DNA damage, which causes depletion of intracellular NAD^+, a substrate of the reaction, reducing intracellular ATP and ultimately resulting in β cell necrosis (249), resulting in the reduction of insulin release and increased glucose levels in animal models (246). Sandler et al. demonstrated that the poly-ADP-ribose polymerase inhibitor, nicotinamide, reversed β cell necrosis, supporting this mechanism (249). To make an animal model with a mild diabetic condition, it has been found that giving nicotinamide to animals prior to STZ injection can counteract the STZ toxicity (250). Thus, animal models with STZ combined nicotinamide injection may attenuate the effect of STZ, inducing only a moderate insulin deficiency along with hyperglycemia. This is analogous to the more advanced state of type II diabetes, when insulin secretion becomes inadequate due to beta cell apoptosis (251). Moreover, if the induced animals are fed a high-fat diet, dyslipidemia and NAFLD will developed, which is also analogous to obesity-induced diabetes (252). However, high doses of STZ injection are thought to be more analogous to type I diabetes, since the islet will be destroyed and animals are insulin deficient more than insulin resistance (253). Another benefit of chemical induction of diabetes is that the chemical induction is inexpensive relative to the use of genetic animal models of diabetes (237). However, diabetic animals induced by STZ are insulin-depleted more than

insulin resistant, which makes it difficult to investigate drugs or therapies that act on the insulin receptor. Finally, there is considerable variability in the degree of diabetes induced by STZ, and the long-term response may be not stable since the chemical damage to beta cells is reversible and spontaneous recovery may occur (254). STZ not only destroys pancreatic islet beta cells but also acts on other tissues, which may interfere with the experiment (255, 256). Thus, STZ combined with nicotinamide to induce diabetes is an appealing approach to produce a model of type II diabetes, as it presents a type II diabetes metabolic phenotype (253). However, the doses of STZ and nicotinamide should be carefully chosen to attain the appropriate severity of diabetes (251).

Similar diabetogenic effects have been obtained using a chemical called alloxan. Alloxan has a high affinity to be reduced to form dialuric acid (257), while dialuric acid can then re-oxidize to alloxan, which forms a redox cycle (258). The superoxide radicals generated from the redox reaction causes the formation of reactive oxygen species (ROS), which destroy the beta cells, resulting in pancreatic cellular necrosis (259). The diabetogenic effects of alloxan in animal models are similar to STZ in that they both act on pancreatic beta cells and thereby reduce insulin secretion.

In a summary, genetic diabetic animal models with hyperphagia are excellent models of obesity-induced diabetes, but are more expensive. However, the lower genetic variation makes it possible to use fewer animals

than with chemically-induced diabetic animal models. The chemically-induced diabetic animal models produce pancreatic beta cell destruction, leading to a greatly diminished ability to secrete insulin, which represents type I diabetes. The use of a combination of STZ and nicotinamide has been promoted as a good model of type II diabetes (252). However, as it is a relatively new model, and its utility is still being investigated.

IV. Hypothesis and objectives

It has been widely accepted that dietary fibers can improve diabetic control, including reducing body weight, hyperglycemia, dyslipidemia, and NAFLD. Many types of dietary fibers have been studied in this regard, including guar gum, pectin, beta-glucans from oats and barley, and synthetic fibers like hydroxypropyl methylcellulose (HPMC). There are few studies that use dietary fibers from fungal sources to investigate the beneficial health effects of fiber intake. Compared to the beta-glucan from yeast and mushrooms, oat-derived beta glucan is extremely difficult to purify, with the most common methods only extracting 60-65% of the beta glucan content (260). This is significant for products such as oat beta-glucan, as the extraction method is critical to the quality of the beta glucan product, and the impurities may play a role in lowering cholesterol and stimulating insulin resistance (261). By contrast, beta-glucans from yeast and mushrooms can be efficiently refined up to 93% purity (262). Further, the beta-glucans from

oats and yeast have different structures, as the linkages between oat-derived beta glucan are β-(1→3) and β-(1→4), whereas in yeast-derived beta glucan the linkages are β-(1→3) and β-(1→6) (261). There are many more studies examining the effect of yeast beta-glucan on immune system development by macrophages than there are on amelioration of diabetes (263). In this study, I have investigated the effect of yeast beta-glucans on glycemic control, liver cholesterol, and fatty liver in a diabetic animal model. My study objectives include the following:

1. To determine whether consumption of yeast beta-glucan will improve glycemic control and reduce insulin resistance in diabetic rats.
2. To determine whether consumption of beta-glucan will reduce liver cholesterol and fatty liver in diabetic rats.

Chapter 2: Methods

Animals and diets

Sixty-five male Wistar rats weighing 81-116 g were purchased in two batches from Harlan Laboratories (Indianapolis, IN). Animals were kept in stainless cages individually. Handling and housing followed the University of Minnesota Policy on Animal Care and Use. All animals were kept in a temperature-controlled room (70 to 73 ℉) with a 12 hour light:dark cycle (light from 6:00 to 18:00).

Rats had free access to water and diet at all times. Prior to induction of diabetes, rats were adapted to a modified AIN-93G control diet for 10 days. Diabetic rats that were selected for inclusion into the study, based on their postprandial glycemic response, were divided into groups and fed either the control diet, a 2% beta-glucan-containing diet, or a 4% beta-glucan-containing diet. Baker Yeast Beta Glucan powder (Wellmune WGP, Dispersible Powder Product, #F3005), a gift from Biothera Co. (Eagan, MN), was used as the source of beta-glucan. The certificate of analysis indicated that the powder contained 86% beta (1,3)-glucan and beta (1,6)-glucan **(Table 2-1)**. The study diets were adjusted to contain 2% and 4% pure beta-glucan. As the beta-glucan powder contained small amounts of digestible macronutrients, the digestible carbohydrate, fat and protein in the two beta-glucan diets were adjusted such that all diets contained equal dietary concentrations of digestible macronutrients **(Table 2-2).** All diets contain 8%

dietary fiber, 10% fat, and 20% protein by weight. The percentage of digestible carbohydrates by weight in each diet was 64.83% in the control diet, 64.66% in the 2% beta glucan diet, and 64.49% in the 4% beta-glucan diet.

Experimental design

The total period of the animal feeding was 52 or 54 days (one-half of rats from each group was harvested on day 52 and one-half of rats was harvested on day 54). On day 4, ten of the total 65 Wistar rats were chosen as the negative control group (non-diabetic control group) whereas the remaining 55 rats were selected to be the diabetic model candidates. The objective was to induce a moderate degree of diabetes, following the procedures described by Szkudelski (251). The protocol used was injection of nicotinamide in 0.9 % sodium chloride solution, (0.09 mg/g body weight), followed 15 min later by injection with streptozotocin dissolved in 0.1 mmol/L citrate buffer (pH=4.5) (0.06 mg/g body weight). The negative controls were given the same amount of nicotinamide in saline as used in the diabetes induction protocol, but then given the same amount of citrate buffer solution (pH=4.5) injections without streptozotocin. The rats were selected for use in the study based on the severity of diabetes, as estimated by a blood glucose tolerance test (GTT) given 4 days after diabetes induction. Rats whose blood glucose levels at either 30 or 60 minutes after administration of a bolus of

glucose (2 g/kg body weight) fell within a range of 270-400 mg/dL were selected for the study. Diabetic rats were then divided into 3 groups, as follows: 10 diabetic rats were feed the AIN-93G diet as a positive control, 9 diabetic rats were fed the diet containing 2% beta glucan, and 9 diabetic rats were feed the diets containing 4% beta glucan. Ten non-diabetic rats (negative control group) were fed the AIN-93G diet. All rats were fed on the control diets for 10 days before assignment to the study diets. Each week, body weight and food intake were recorded. On day 36 and day 37, 24-hour urine collections were made, using a metal metabolic cage with a wire mesh floor, as described by Haas (264). Urine was collected into a beaker placed under the cage of each animal. All animals had free access to water during the collection. After collection, urine was centrifuged at 5 ℃ at 2800 *g* for 12 minutes to separate urine from food residues and the total 24-hour urine volume was determined. Five mL of urine from each animal was stored at -20 ^{o} C for glucose and thiobarbituric acid reactive substances (TBARS) assays. On day 45 and day 46, an oral glucose tolerance test (GTT) was conducted. Blood samples (approx.150 µL) were collected from the saphenous vein during fasting and every 30 minutes after gavage of a bolus of glucose (2 g/kg body weight) for 2 hours and placed into tubes containing 10 EDTA (2.0 mg EDTA/mL). Blood was centrifuged at 14,000 rpm for 10 min and plasma stored at -80 ^{o} C until analysis. (See Appendices A for details). On day 47 and day 48, a two-day fecal collection was made. Feces were dried, weighed

and stored in -20 º C until analyzed. One day 51, a pyruvate tolerance test (PTT) was conducted to assess hepatic gluconeogenesis. Sodium pyruvate dissolved in saline was injected intraperitoneally (2.0 g sodium pyruvate /kg body weight) and blood glucose was measured on the upper side of the tail using an AlphaTRAK® blood glucose monitor. (See Appendix B for details.) On day 52 and 54, rats were deprived of diet for 14 hours, beginning in the evening, then given 3 g of their respective diet the next morning. The quantity of the meal consumed was recorded. Two-hour after feeding, the rats were anesthetized by isoflurane, blood were collected into an EDTA-containing syringe (11-14 mg/ syringe) by cardiac puncture, and tissues were harvested. Blood samples were centrifuged at 3000 *g* for 15 minutes at 4 º C and the plasma and packed cells were collected and store in -80 º C until analysis. Liver, both kidneys, and epididymal fat pads were collected, weighed and frozen in liquid nitrogen. All organs were stored at -80 º C. The small intestines were excised, intestinal contents were collected by finger stripping, and the contents were stored on ice until being centrifuged at 30,000 *g* for 30 min at 37 °C using a JA 20.1 Rotor (Beckman Instruments, Spinco Division, Palo Alto, CA). The intestinal contents viscosity was measured the same day after collected, as described below.

Glycemic Analysis

Plasma glucose concentration was determined enzymatically using a glucose

oxidase-peroxidase reagent (265). See Appendices C for details. The incremental area under the curve (iAUC) was calculated for the postprandial glycemic response.

The incremental area under the glucose curve (iAUC) was also used to calculate from pyruvate tolerance test to estimate the postprandial hepatic gluconeogenesis response.

Urinary glucose concentration was assayed followed the enzymatic method of Morin and Prox using the glucose oxidase-peroxidase reagent (265). See Appendices C for details.

Glycated hemoglobin

Glycated hemoglobin indicates the average blood glucose levels over the last 3 months. Frozen packed blood cells were thawed and an aliquot (50 μL) of packed red blood cells was assayed according to the affinity-chromatographic method of Klenk (266), using a commercial kit (GLYCO-Tek Affinity Column Kit, Helena Laboratories, Beaumont, TX). Normal GHb and abnormal elevated GHb calibrators were measured to ensure the accuracy of the assay. Results were expressed as the percentage of glycated hemoglobin. See Appendix D for details.

Hepatic lipids and cholesterol

One gram of liver tissue was homogenized with a 2:1 chloroform-

methanol solution and purified according to the method of Folch et al. (267). The extracts were dried under nitrogen and the quantity of liver lipids determined gravimetrically. The extract was then reconstituted with 2:1 chloroform-methanol for hepatic cholesterol analysis. An aliquot of the reconstituted extract was mixed with 0.1% Triton X-100 in acetone and cholesterol measured enzymatically as described by Warnick et al. (268). Hepatic cholesterol concentration was quantitated by comparison to a cholesterol standard of known concentration. See Appendixes E-F for details.

Small intestinal contents viscosity

The supernatants of small intestinal contents after centrifuging were collected and the supernatants were brought to 37 ° C. Viscosity was measured using a Wells-Brookfield cone/plate viscometer (model RVT, Brookfield Engineering, Stoughton, MA) between 1.15 and 230 s-1 shear rates. Viscosity was calculated by plotting viscosity versus shear rate on a log-log scale, and extrapolating to a common shear rate of 23 sec^{-1}. See Appendix G for details.

Fecal beta-glucan analysis

Two day fecal samples were collected on day 47 and day 48 and the samples were pooled, dried, weighed, and milled by a mortar and a pestle for further analysis. The milled fecal samples were sieved to pass a 1.0 mm

screen and an aliquot of the fecal sample was used to determine beta-glucan, using a commercial kit (Yeast & Mushroom Beta-glucan Assay kit, Megazyme Inc., Ireland), which is based on the acidic and enzymatic method developed by Park and Ikeagaki (269). The milled fecal samples were solubilized in ice cold sulfuric acid for 2 hours with vigorous vortexing to ensure dissolution of the beta-glucan. The samples were then placed in a boiling water bath for 2 hours and the boiled fecal samples were then reconstituted with potassium hydroxide and sodium acetate buffer. The samples were then centrifuged at 1,500 *g* for 10 min and the supernatant collected. The supernatants were then incubated for 60 minutes at 40° C with exo-1,3-β-glucanase (20 U/mL) and β-glucosidase (4 U/mL) to hydrolyze the beta-glucan to D-glucose. An enzyme cocktail (GOPOD reagent) was added to hydrolyze samples and measure the resulting glucose spectrophotometrically. Total glucan concentration in the samples was determined by comparison to a known D-glucose standard concentration. Another aliquot of 100 mg milled fecal samples was used to determine the amount of alpha-glucan (i.e. starch, phytoglycogen). The milled fecal samples were dissolved in potassium hydroxide and incubated at 40 ° C for 30 minutes with amyloglucosidase (1,630 U/mL) and invertase (500 U/mL) to specifically convert the alpha-glucan to glucose. The samples were then mixed with the GOPOD reagent and glucose measured spectrophotometrically. The results were compared to a known D-glucose

standard concentration to determine the alpha-glucan in the animals' feces. The total beta-glucan in the fecal sample was then calculated by the difference between the total glucan and the alpha-glucan content of each sample. The quantity of beta-glucan fermented was estimated as the difference between total beta-glucan intake per day, determined by multiplying food intake by the beta-glucan percentage of their respective diet, and beta-glucan excreted per day. See Appendices H for details.

Statistical analysis

Data is reported as means ± SEM. Differences between different groups were analyzed by one-way analysis of variance (ANOVA). $p \geqslant 0.05$ was considered as indicating no statistically significant difference. When the ANOVA indicated a statistically significant difference, the differences among groups were analyzed by Duncan's multiple range test (270).

Table 2-1

Certificate of analysis of Baker's Yeast Beta glucan[1]

Attribute	Results	Method of Analysis
Gluco polysaccharide (Beta 1,3/1,6)[2]	86%	Assay per FCC beta glucan from Baker's Yeast Monograph
Carbohydrates	85.44%	By calculation
Protein	2.59%	AOAC 990.03
Fat	4.85%	AOAC 989.05

[1]The certificates of analysis was provided by Biothera Company in Eagan, MN. The product name is "Wellmune WGP® Dispersible Powder". Common name is Baker's Yeast Beta Glucan.

[2] The alternative name of Baker's Yeast Beta Glucan is Gluco Polysaccharide.

Table 2-2

Diet composition[1]

Diet Ingredients	Control diet	2% Beta-Glucan	4% Beta-Glucan
		g/kg diet	
Beta-glucan[2]	0	23.408	46.816
Sucrose	100	100	100
Maltodextrin	132	132	132
Corn starch	336.286	334.584	332.882
Soybean Oil	100	98.900	97.800
Casein	200	199.394	198.787
Cellulose	80	60	40
Mineral mix	35	35	35
Vitamin mix	10	10	10
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	2.5
Cholesterol	1.2	1.20	1.200
TBHQ	0.014	0.014	0.014

[1]Modified from the AIN-93G diets. Dietary treatments are as followed: Control diet = AIN-93G diet, 2% diet = Modified AIN-93G diet with 2% beta glucan, 4% diet = Modified AIN-93G diet with 4% beta glucan.

[2]The beta-glucan powder contained 86% Beta-glucan.

Chapter 3: Results

Body Weight and Food Intakes

Weekly body weights are presented in Figure 3-1. There were no significant differences among the four groups in initial body weights. After induction of diabetes and the initiation of feeding the experimental diets, the negative control group (non-diabetic rats) showed significantly greater weight gains than the diabetic groups through the last week of feeding. Throughout the seven weeks of feeding, there were no significant difference in body weights among the positive control (diabetic rats fed on the control diet), the 2% beta-glucan and the 4% beta-glucan groups. Food intakes were measured weekly from the second week to the week before the end of the study (Table 3-1). At the second week of feeding, the negative control group showed a significantly lower daily food intake than the positive control group and the 2% beta-glucan group. However, there was no significant difference in daily food intake between negative control group and 4% beta-glucan group. The 4% beta-glucan group also showed no significant difference from positive control and the 2% beta-glucan group. From the third week to the sixth week, the negative control group had significantly lower food intake than all the other groups. At the third and the fourth weeks, no significant differences were found among the diabetic groups. Starting from the fifth week, the 2% beta-glucan group shows significantly greater food intakes than the 4% beta-glucan group. There were no significant differences between the positive

control group and the 2% beta-glucan group or between the positive control and the 4% beta-glucan group in the fifth week. In the sixth week, the 2% beta-glucan group had a greater food intake than all other groups, and no significant difference was found between the 4% beta-glucan group and the positive control group.

Urine volume and tissue weights

The negative control group had significantly lower 24 hours urinary output than the positive control, the 2% beta-glucan or the 4% beta-glucan group (Table 3-2). No significant differences were found among any of the diabetic groups. The negative control group had significant greater liver weight and epididymal fat pad weight than the diabetic groups. Significantly lower kidney weight was found in the negative control group than the diabetic groups. The liver weight, kidney weights, and the epididymal fat pad weights were equivalent among the diabetic groups.

Glycated hemoglobin, glucose tolerance test, pyruvate tolerance test, and urinary glucose

The negative control group showed significantly lower percent glycated hemoglobin than the diabetic rats from the positive control group, the 2% beta-glucan group and 4% beta-glucan groups (Figure 3-2), indicating successful induction of diabetes in all diabetic groups, to approximately the

same degree. However, no significant difference was observed among the three diabetic groups. The negative control group also showed significantly lower fasting blood glucose levels, as well as lower glucose at 30, 60, 90, and 120 minutes after the start of the oral glucose tolerance test **(figure 3-3)**. The positive control group, 2% beta-glucan group, and 4% beta-glucan group showed statistically equivalent blood glucose levels at fasting and at 30, 60, and 90 minutes after the start of the glucose tolerance test. The diabetic rats fed the 4% beta-glucan diet showed slightly but significantly higher levels of blood glucose at 120 minutes after oral glucose tolerance test than the diabetic rats fed on basal diet. No significant differences were observed at 120 minutes after oral glucose tolerance test between the 4% beta-glucan group and the 2% beta-glucan group or between the positive group and the 2% beta-glucan group. To better estimate the glycemic responses during the glucose tolerance test, the incremental area under the curve (iAUC) was calculated from the blood glucose levels. The iAUC of the negative control group was significantly lower than all the diabetic animal groups (**figure 3-4**). No significant differences in the iAUC were found among the three diabetic rat groups. A pyruvate tolerance test was conducted to estimate the degree of hepatic gluconeogenesis. The negative control group had significantly lower blood glucose levels before pyruvate administration and 30, 60, 90, and 120 minutes afterwards (**Figure 3-5**). The diabetic groups fed on the control diet, the 2% beta-glucan diet, and the 4% beta-glucan diet had

statistically equivalent levels of blood glucose before and at all points after the pyruvate tolerance test. The non-diabetic negative control group had significantly lower glucose iAUC than the diabetic groups. Further, no significant differences in the iAUC were found among the diabetic groups (**Figure 3-6**).

High plasma glucose levels may result in glucose wasting into the urine, due to exceeding the renal threshold for plasma glucose. All diabetic groups had a large and statistically significantly greater 24 hr urinary glucose output than the non-diabetic rats of the negative control group, but no significant difference in urinary glucose output was observed among the diabetic groups **(Figure 3-7)**.

Hepatic lipids

There was no significant difference in hepatic fat concentration among the negative control group and the diabetic rats of the positive control group, diabetic rats of 2% beta-glucan group, and diabetic rats of the 4% beta-glucan group (**Figure 3-8**). However, a large variation was observed in the hepatic fat concentrations of the 4% beta-glucan group. In contrast, total liver fat was statistically equivalent among all 4 groups (**Table 3-2**). The groups fed the beta-glucan-containing diets both had a lower hepatic cholesterol concentration than the control diet-fed diabetic group, which had the greatest cholesterol concentration of any group (**Figure 3-9**). The hepatic cholesterol

concentrations were reduced by 33% in diabetic rats with 2% beta-glucan in the diet, and was reduced by 70% in diabetic rats with 4% beta-glucan in the diet compared to the positive control group. No significant differences were observed between the negative control group and either the 2% beta-glucan group or the 4% beta-glucan group. However, the 4% beta-glucan group did have a significantly lower hepatic cholesterol concentration than the 2% beta-glucan group. The pattern was similar when total liver cholesterol was examined (**Figure 3-10**). Total liver cholesterol was significantly greater in the positive control group compared to all other groups. Total liver cholesterol in the 2% beta-glucan group was 35% lower and the 4% beta glucan groups was 70% lower compared to the positive control group. Total liver cholesterol of the negative control group and the 2% beta-glucan group did not differ. Total liver cholesterol in the 4% beta-glucan group was significantly lower than all other groups.

Viscosity and fermentability of yeast-derived beta-glucan

Small intestinal contents viscosity was very low and did not differ among any of the groups (**Figure 3-11**). Fermentation of the yeast-derived beta-glucan was confirmed. Rats fed the 2% beta-glucan diet had a lower percentage of the consumed beta-glucan fermented than rats fed the 4% beta-glucan diet, a difference that was statistically significant (**Figure 3-12**). Only approximately 10% of the beta-glucan consumed in the 2% beta-glucan group was

fermented, whereas approximately 50% of the beta-glucan consumed by the 4% beta-glucan group was fermented.

Table 3-1

Food Intakes of non-diabetic rats (negative control group), diabetic rats with basal diet, diabetic rats with 2% beta-glucan diet and diabetic rats with 4% beta-glucan diet for 6-7 weeks[1]

Group	*Week 2 (g/d)*	*Week 3 (g/d)*	*Week 4 (g/d)*	*Week 5 (g/d)*	*Week 6 (g/d)*
Negative Control (non-diabetic)	19.2±0.6^{b}	28.5±3.2^{b}	40.3±4.7^{b}	18.5±1.2^{c}	17.8±1.7^{c}
Positive Control (diabetic)	27.4±0.9^{a}	34.1±2.0^{a}	67.5±1.8^{a}	44.4±5.6^{ab}	41.9±5.3^{b}
2% beta-glucan	27.8±2.5^{a}	31.9±1.3^{a}	66.5±3.0^{a}	58.9±7.5^{a}	65.7±7.2^{a}
4% beta-glucan	22.3±1.7^{ab}	38.9±2.5^{a}	63.0±3.8^{a}	38.5±5.8^{b}	41.5±4.6^{b}

[1] Values are present as means±SEM, n=5-7. Values with different superscripts in a column are significant different ($p<0.05$)

Table 3-2

Twenty-four hour urine output, liver weight, kidney weight, epididymal fat pads weight, and total hepatic fat[1][2][3]

Group	*24-hours urine output (mL)*	*Liver weight (g)*	*Kidneys weight[2](g)*	*Epididymal fat pads weight[3] (g)*	*Total hepatic fat (g)*
Negative Control (non-diabetic)	3.1±1.3b	13.7±0.9a	2.38±0.07b	4.76±0.56a	1.93±0.28
Positive Control (diabetic)	150.5±16.3a	10.9±0.8b	2.91±0.12a	1.74±0.24b	1.60±0.28
2% beta-glucan	134.9±15.3a	10.8±0.3b	2.98±0.07a	1.54±0.12b	1.18±0.11
4% beta-glucan	131.7±10.7a	10.5±1.1b	2.94±0.24a	1.43±0.27b	1.50±0.47

[1] Values are present as means±SEM, n=5-7. Values with different superscripts in a column are significant different ($p<0.05$).

[2] Two kidneys were collected and weighed together from each animal model

[3]Two epididymal fat pads from each animal model were collected and weighed

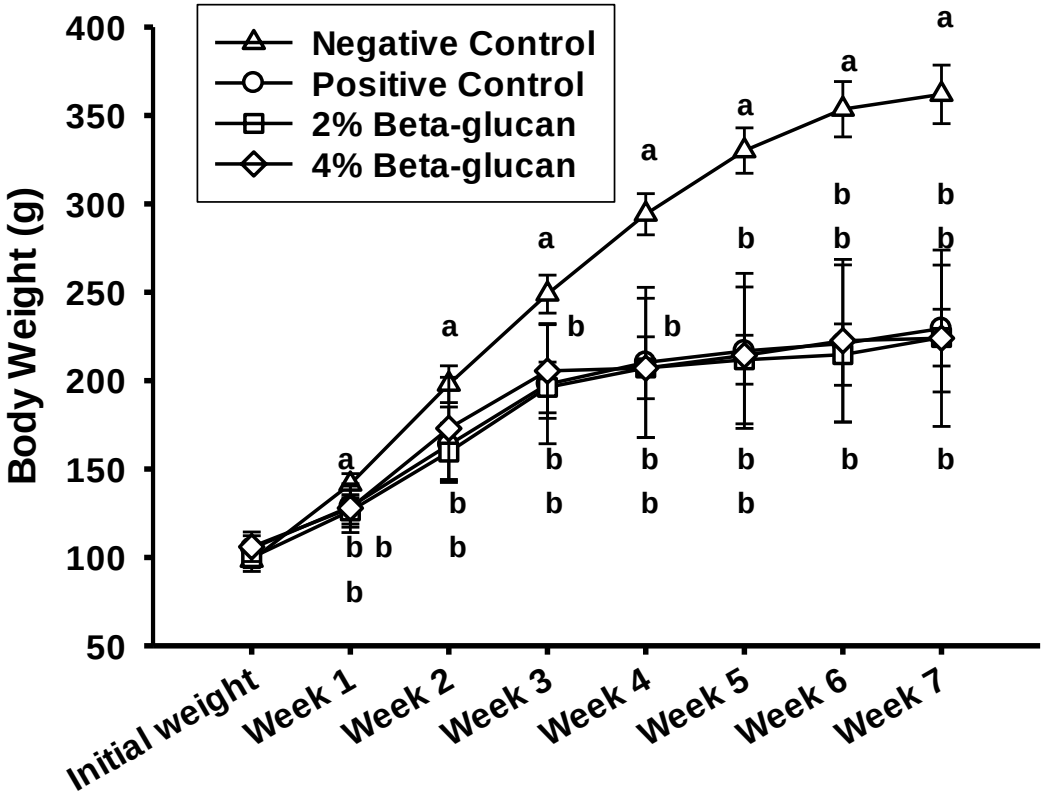


Figure 3-1. Body weights (g) of non-diabetic rats fed the basal diet (Negative Control), diabetic rats fed the basal diet (Positive Control), diabetic rats fed the 2% beta-glucan and diabetic rats fed the 4% beta-glucan. Values are present as means ± SEM, n=5-7. Values with different superscripts within a week are significant different ($p<0.05$).

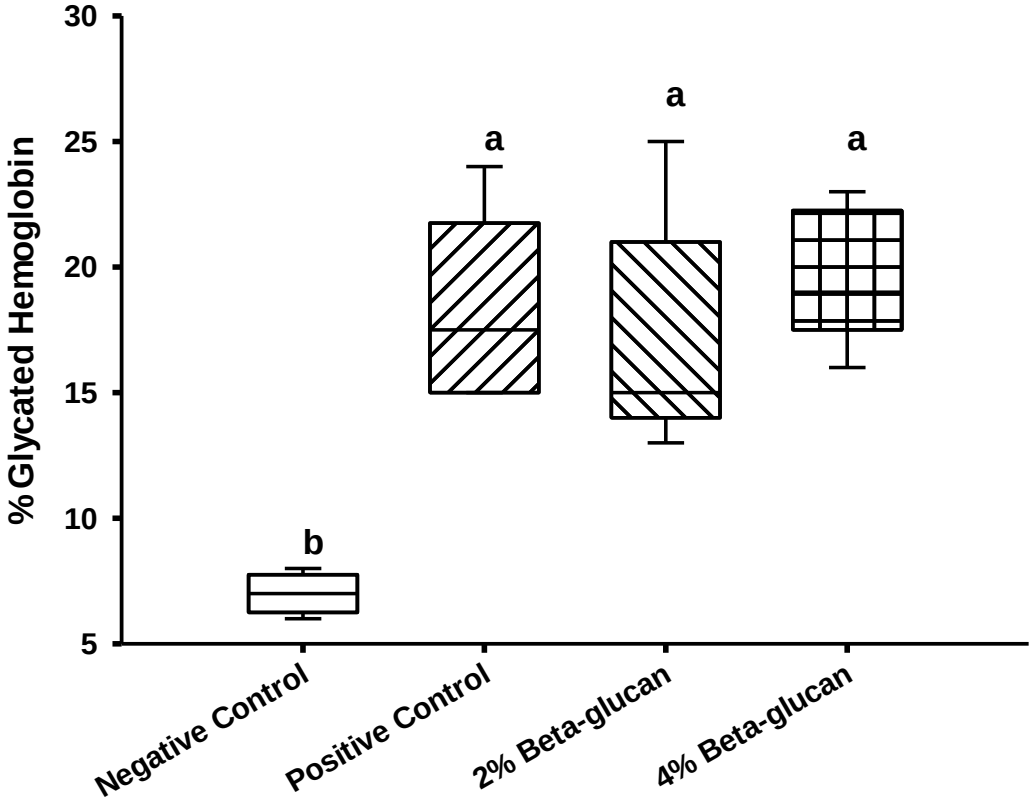


Figure 3-2. Percentage glycated hemoglobin in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7. Values with different superscripts are significantly different ($p<0.05$).

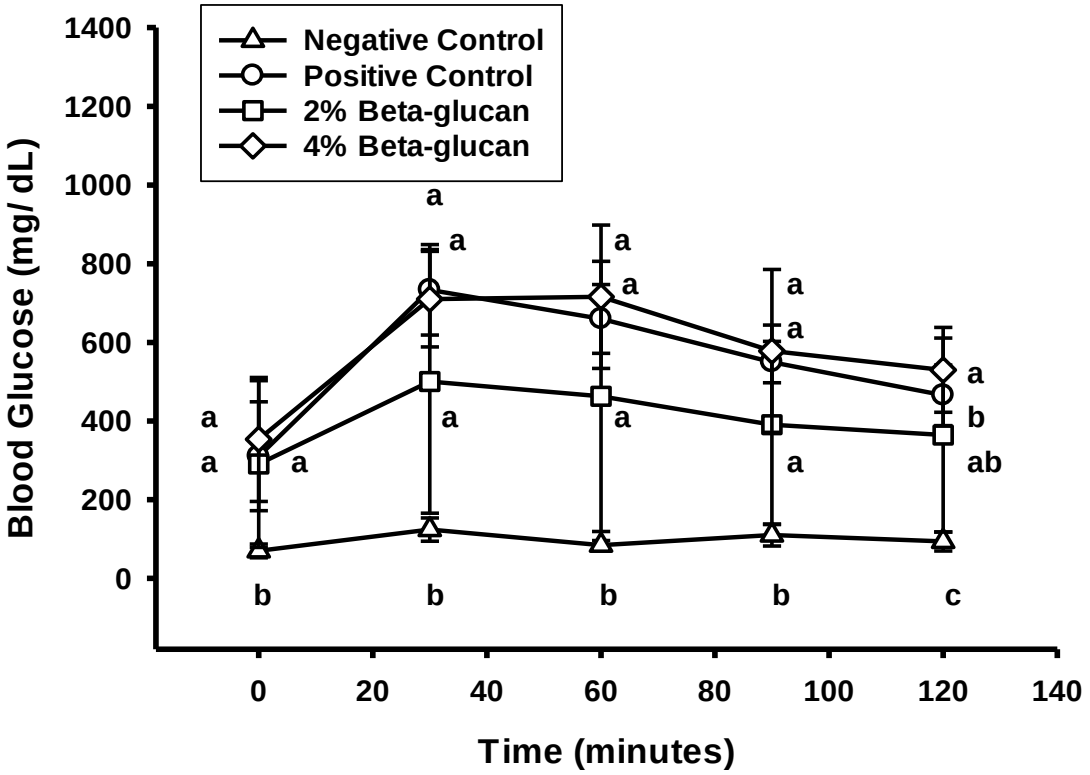


Figure 3-3. Blood glucose levels (mg / dL) of initial time, 30 minutes, 60 minutes, 90 minutes and 120 minutes after oral glucose tolerance test in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7. Values with different superscripts within a time point are significantly different ($p<0.05$).

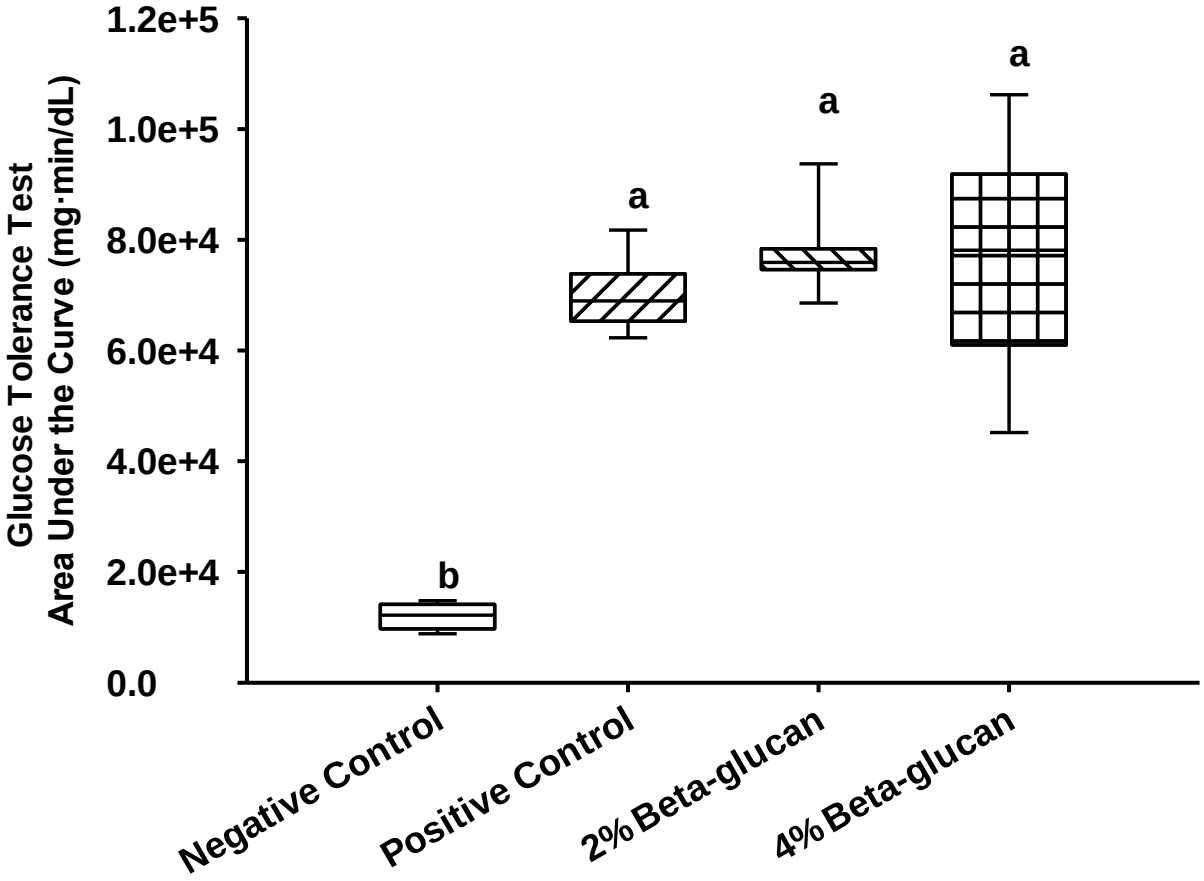


Figure 3-4. Area under the curve of the oral glucose tolerance test (mg× minutes/dL) in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7. Values with different superscripts are significantly different ($p<0.05$).

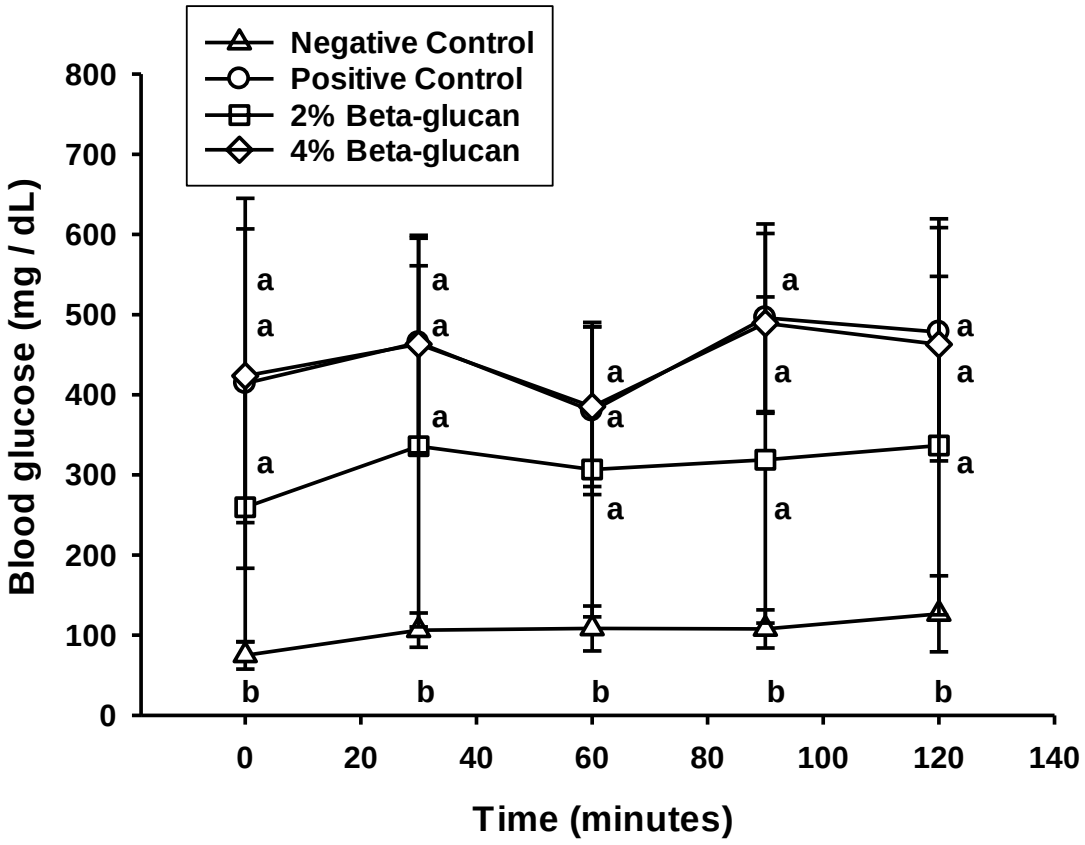


Figure 3-5. Blood glucose levels (mg/dL) of initial time, 30 minutes, 60 minutes, 90 minutes and 120 minutes after pyruvate tolerance test in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7.

Values with different superscripts at a time point are significantly different ($p<0.05$).

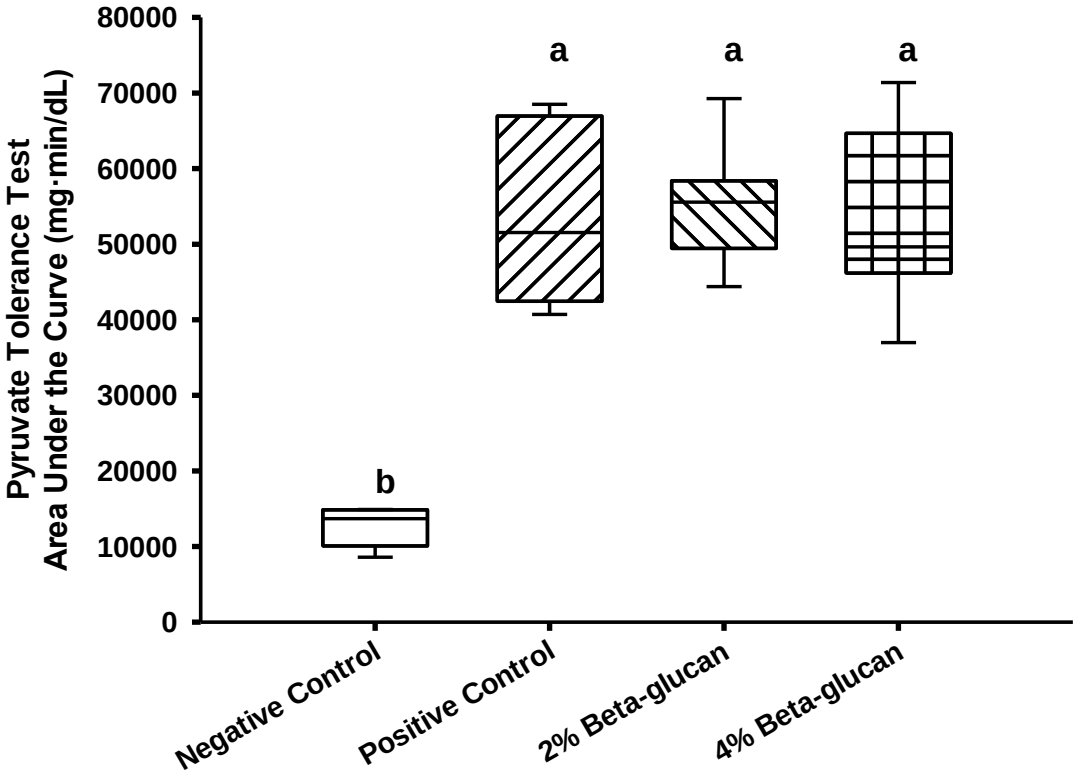


Figure 3-6. Area under the curve of pyruvate tolerance test in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7. Values with different superscripts are significantly different ($p<0.05$).

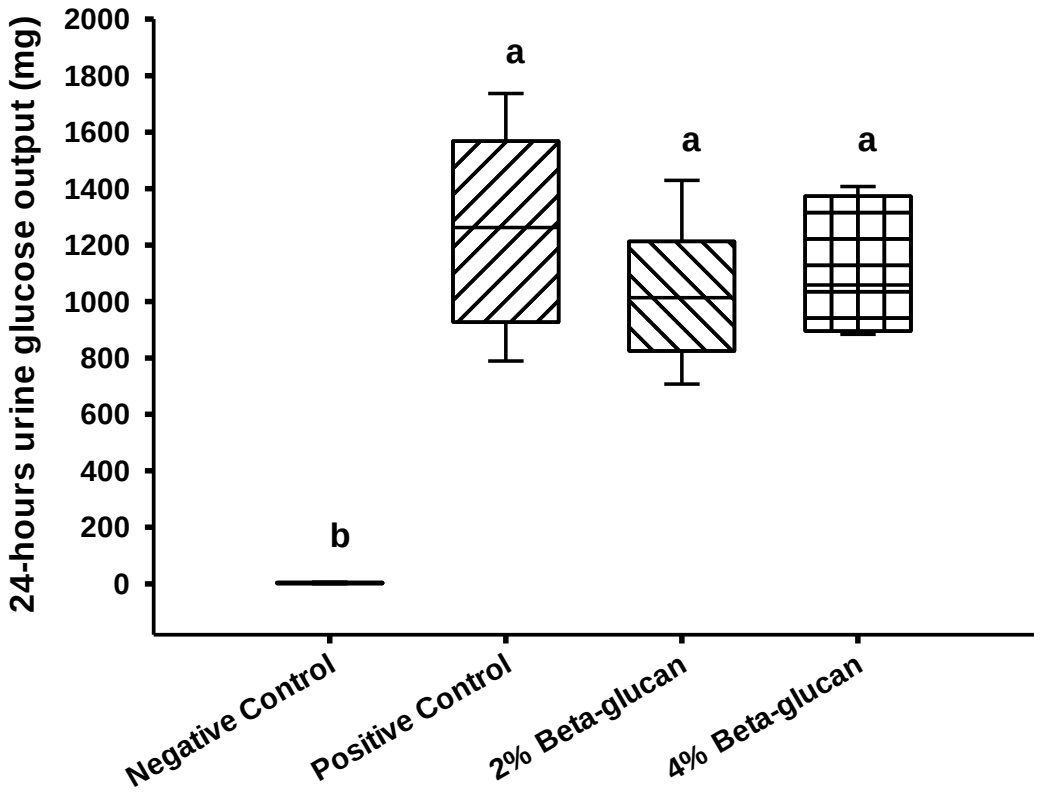


Figure 3-7. Twenty-four hour urinary glucose output in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7. Values with different superscripts are significantly different ($p<0.05$).

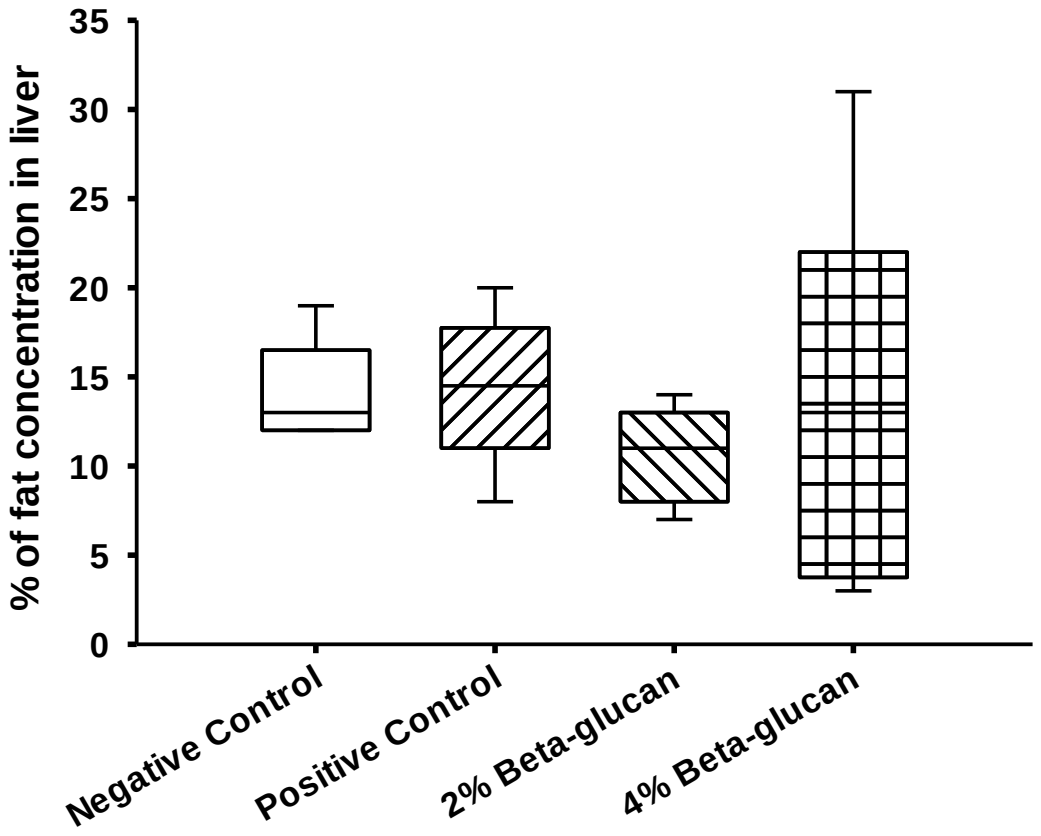


Figure 3-8. Percentage of lipid in liver in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7.

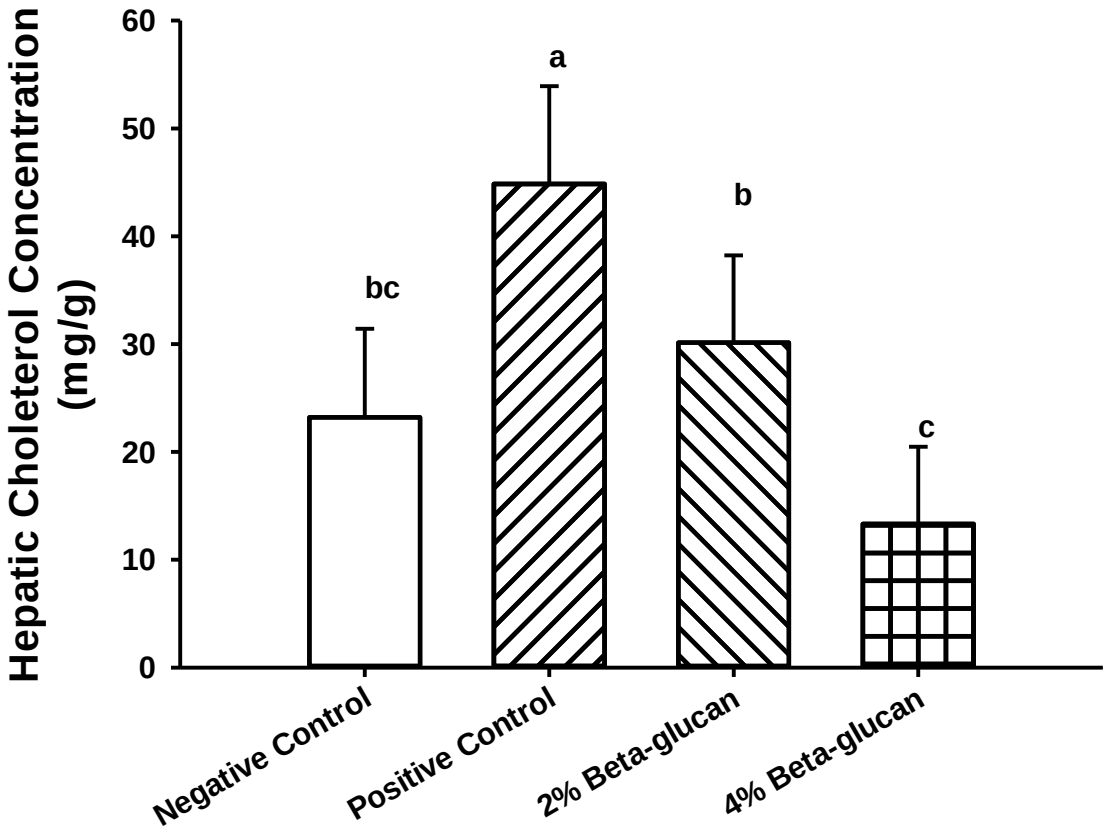


Figure 3-9. Hepatic cholesterol concentration in non-diabetic rats fed on basal diet (negative control), diabetic rats fed on basal diet (positive control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as mean ± SEM. N=5-7. Groups not sharing the same letter are significantly different ($p<0.05$).

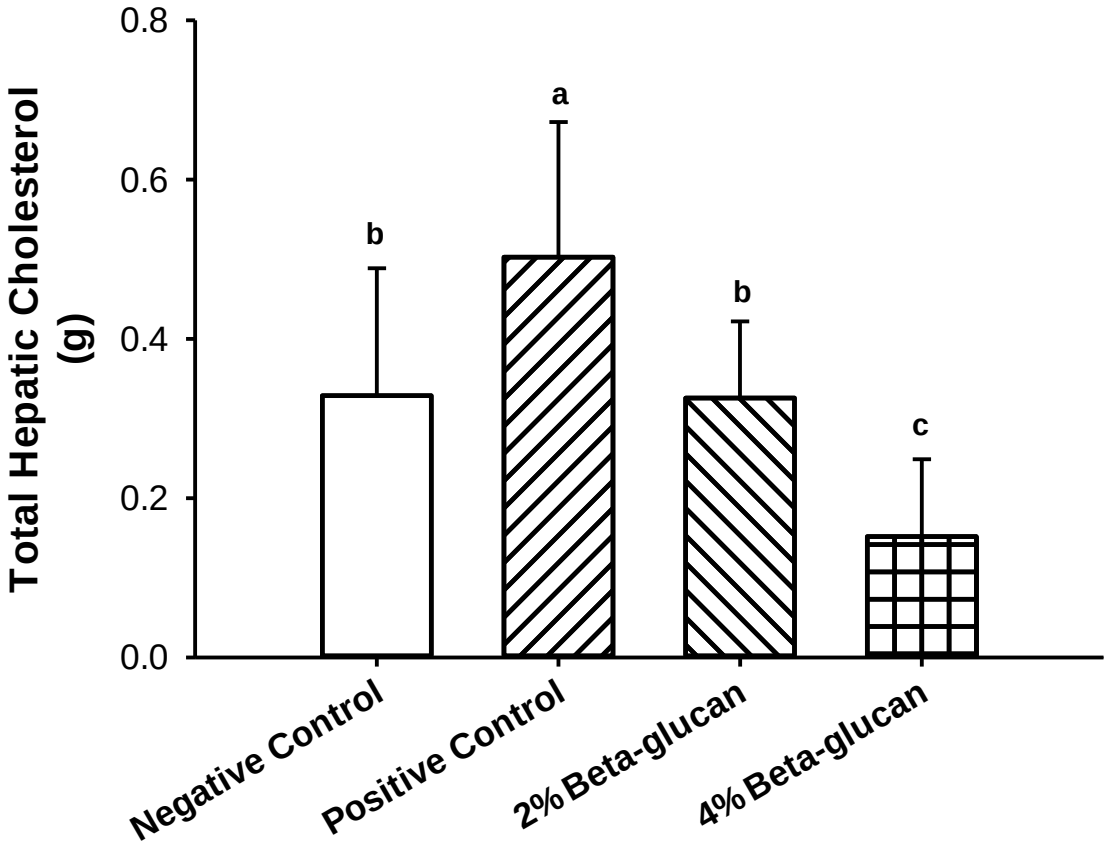


Figure 3-10. Total liver cholesterol in non-diabetic rats fed on basal diet (negative control), diabetic rats fed on basal diet (positive control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as mean ± SEM. N=5-7. Groups not sharing the same letter are significantly different ($p<0.05$).

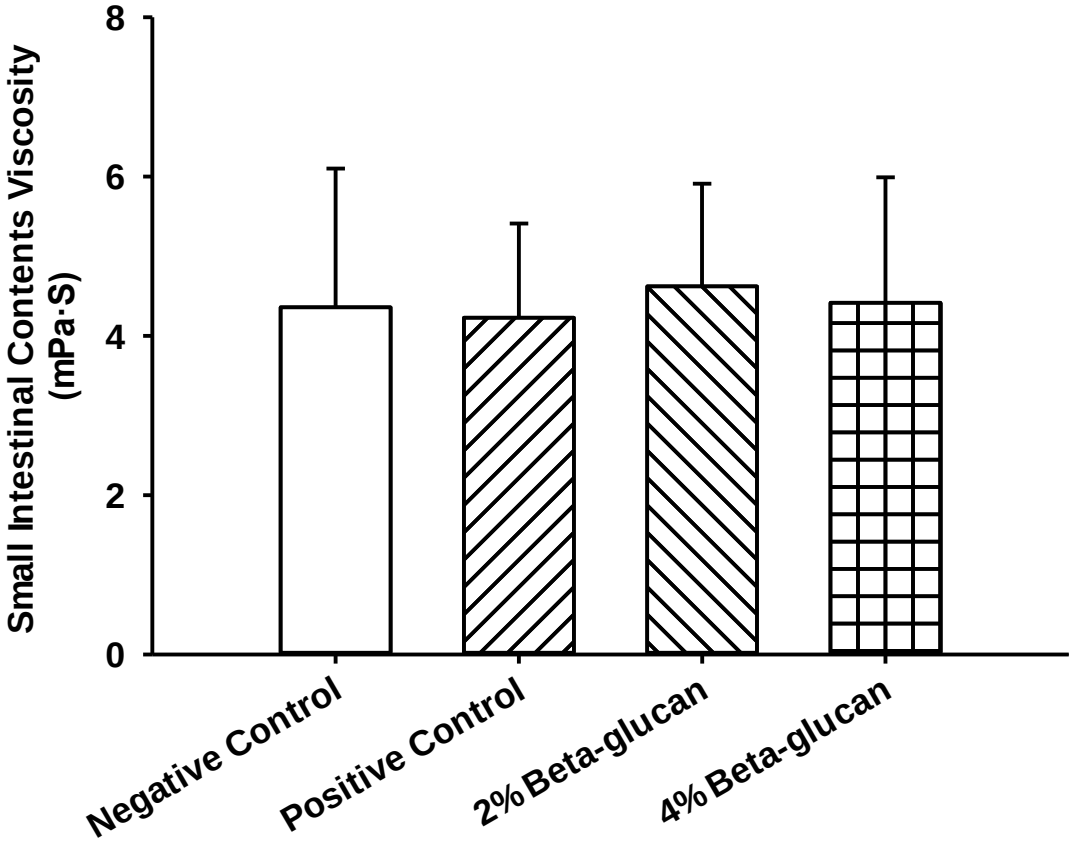


Figure 3-11. Small intestine contents viscosity in non-diabetic rats fed on basal diet (Negative Control), diabetic rats fed on basal diet (Positive Control), diabetic rats fed on 2% beta-glucan and diabetic rats fed on 4% beta-glucan. Values are presented as means ± SEM, n=5-7.

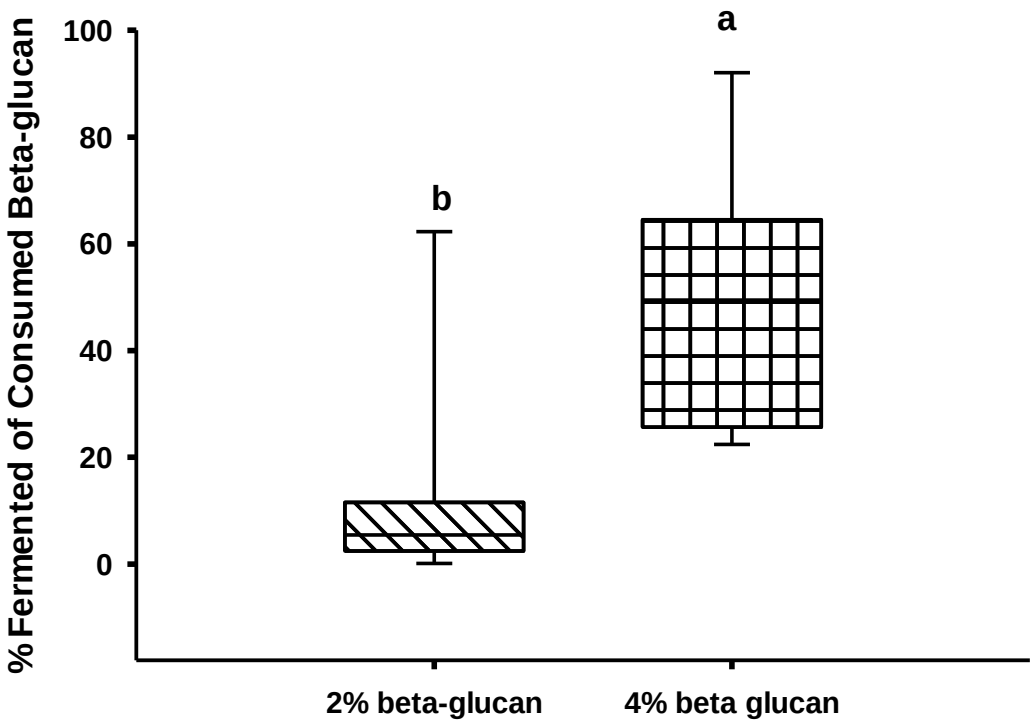


Figure 3-12. Percent of consumed beta-glucan that was fermented in diabetic rats fed the 2% beta-glucan or 4% beta-glucan diets. Values are presented as means ± SEM, n=5-7. Values with different superscripts in a column are significantly different ($p<0.05$).

Chapter 4: Discussion

Diabetes mellitus was ranked as the 7th leading cause of death in 2012 (1). Although there is no specific recommendation for dietary fiber for diabetic patients, the 2010 USDA dietary guidelines recommended consuming dietary fiber naturally present in foods to improve type II diabetes (271). Of the many serious complications accompanying diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) is one of the most common, but it is also a highly treatable health problem. Intake of certain types of dietary fiber has been shown to be effective in reducing the postprandial glucose response, glycated hemoglobin percentage and LDL cholesterol concentration, and improving the glycemic control in diabetes and diabetic complications (152, 272). Besides the improvement in glycemic control, certain dietary fibers also reduced fatty liver in insulin resistant animal models (18, 171, 178, 181). In most studies that demonstrated the beneficial effects of dietary fibers on insulin resistance and fatty liver progression, it is the viscous, soluble fiber from cereal sources that are effective (167, 178, 181). Fewer studies have examined the effect of non-viscous or insoluble fibers on the glycemic response or lipid profile in subjects with insulin resistance (11, 273, 274). Among these studies, the effect of non-viscous fibers is inconsistent. Some studies demonstrated that non-viscous fibers can reduce plasma lipids, slow down the progression of NAFLD in diabetic models (13), and improve glycemic control in insulin-resistant animal models (224, 275, 276). However, other studies indicate that the glycemic control and cholesterol lowering effects largely depends on the viscosity of the dietary fiber, and that non-viscous fiber has little effect on improving glycemic responses or reducing NAFLD progression (11, 274). My study was designed to testify whether yeast beta-glucan, a non-viscous, fermentable fiber, can improve glycemic control and reduce progression of non-alcoholic fatty liver disease in a diabetic animal model.

My study was intended to create a type II diabetic animal model by using the combination of streptozotocin (STZ) and nicotinamide (NA). Although STZ is used to create type I diabetes by inducing beta cell necrosis and eventually leads to insulin exhaustion, recent studies proposed that a

nicotinamide injection prior to STZ injection may delay the pancreatic beta cell apoptosis and partially rescue beta cells to maintain some insulin secretion, thereby inducing a relatively mild hyperglycemia, and creating a type II diabetic animal model (251, 277). However, based on the high final glycated hemoglobin values and high fasting blood glucoses, our animals appeared to be exhibiting type I diabetes rather than type II diabetes. This was further confirmed by the high blood glucose levels in two hours after an oral glucose tolerance test and the extremely high glucose levels during the pyruvate tolerance test. The reason for this may have been the age of our animals, as some studies have suggested that age of the rats and their nutrition status will affect the induction of diabetes using STZ-NA (251). Younger aged rats tend to be less sensitive to STZ with the same dose of STZ, and therefore are more likely to develop type II diabetes than type I diabetes (278). Although the study that the dose of STZ was based on did not specify the age of rats when STZ injection occurred, our unsuccessful induction of type II diabetic animal models might be attributed to STZ injection at an older age.

As viscosity and fermentability have been considered as two predominant characteristics of dietary fibers in predicting its physiological effects, we investigated the small intestinal contents viscosity and large intestinal fermentation to verify these characteristics in the yeast derived beta-glucan used in our study. The yeast beta-glucan used in this study has 86% purity, with minor proportions of fat and protein. Since the small intestinal content viscosities were statistically equivalent in all groups, this confirmed that yeast-derived beta-glucan has essentially no viscosity. This result is consistent with previous studies by Yoshida et al. (279) and Nicolosi et al. (280). Yoshida used the cell wall of yeast *Kluyveromyces marxianus* as a beta-glucan source while Nicolosi used the spent yeast from bakeries. In both cases, the yeast derived beta-glucan had very low in vitro solubility and viscosity.

We also investigated the fermentability of the yeast-derived beta-glucan by measuring beta-glucan excretion in the feces compared to beta-glucan

intake. The results demonstrated that yeast beta-glucan were partially fermented, but the degree of fermentation was highly variable, ranging from 0.2% up to 90%. This confirmed that yeast beta-glucan is partially fermentable in the gut, which is consistent with previous studies conducted by Yoshida et al. (279), who reported that yeast beta-glucan consumption increased fermentation by-products, short chain fatty acids, in the large intestinal contents. In the present study, in the 4% beta-glucan group, approximately 50% of the beta-glucan was fermented, which was significantly greater than the average of 10% beta-glucan fermented in the 2% beta-glucan group. Our results indicate that greater beta-glucan intake resulted in a greater proportion of it being fermented, suggesting an induction of the fermentation process by beta-glucan. The most common factors influencing fiber fermentation are solubility and the viscosity of the fiber, the available surface and the bulk volume (137, 147, 281). However, yeast beta-glucan is insoluble, with no viscosity, and thus the available surface and bulk volume will not change with consumption. It is also worthy of mention that the fermentation rate may be modulated by individual differences due to different compositions of microflora with a broad spectrum of enzyme activities (282). This suggestion is consistent with our findings that the animal models in both 2% beta-glucan group and 4% beta-glucan group had large variations in the beta-glucan fermentation proportions.

The effectiveness of diabetic induction in diabetic groups was confirmed by the increase in multiple measures of glycemic control, including glycated hemoglobin and urinary glucose excretion. However, no significant differences were observed in any measure of glycemic control between the diabetic rats with no yeast beta-glucan intakes and diabetic rats consuming either 2% or 4% yeast beta-glucan. Our results suggest that consumption of yeast-derived beta-glucan does not improve glycemic control in diabetic rodents. These results are not consistent with the studies of Vieiria et al. (222), who reported a 30% reduction in the postprandial glycemic response, or Silva et al. found significantly lower fasting blood glucose levels (221) in STZ-treated diabetic rats administered yeast derived beta-glucan orally in a

solution. However, a number of studies indicate that viscosity of fiber is the main predictor whether a fiber will reduce postprandial glycemia (283, 284). For example, Brenelli et al. used three different dietary fibers with a similar initial viscosity (guar gum, pectin, and carboxymethylcellulose). The fibers were then put through an in vitro “digestion” and tested for their effects on the postprandial glucose response after a meal in humans (285). The result showed that the “after digestion” viscosity of the fiber down-regulate the postprandial glucose concentration. Guar gum, with highest “after digestion” viscosity, had the lowest postprandial glucose levels. And pectin, showed no significant effects in lowering postprandial glucose with lowest “after digestion” viscosity. This study suggests that viscosity of a fiber in the gastrointestinal tract is the factor affecting glycemic control. Another study used two different methods (enzymatically method versus aqueous method) to process oat beta-glucan (133). The enzymatically processed beta-glucan resulted in a product with a greater viscosity than the aqueous method. The postprandial glucose response in humans after consuming containing these two products, in a beverage, showed that the enzymatically method processed product attenuated the postprandial blood glucose response much more than the aqueous product beverage. This study demonstrated that even with the same product, by changing the processing method to preserve fiber’s differences in viscosity of the same material, beta-glucans in this case, results in different postprandial glycemia. In another human study, healthy non-obese males were fed meals containing 100 g carbohydrate with either 10 g of an insoluble fiber mixture of arabinogalactan and arabinan, or 10 g mixture of the soluble fiber pectin. The subjects with pectin in their test meals had a significant reduction in postprandial blood glucose, circulating insulin and gastric inhibitory polypeptide (GIP) levels, a hormone that induces insulin secretion to decrease blood glucose levels, compared to subjects with only insoluble fiber (286). These results indicated that an insoluble fiber, with no viscosity, had little effects on reducing postprandial glycemic responses. Together, these studies indicate that viscosity of the dietary fiber is the characteristic that is responsible for the reduction in postprandial blood

glucose, which is consistent with our findings that yeast beta-glucan, which imparted no intestinal contents viscosity, did not improve glycemic control in diabetic rodents.

Although yeast beta-glucan did not improve glycemic control, it did lower liver cholesterol. The diabetic rats from positive control group showed significantly greater liver cholesterol concentration than the non-diabetic rats from the negative control group. However, diabetic rats from 2% beta-glucan group had significantly lower concentrations of hepatic cholesterol compared to the positive control group, a concentration that was similar to concentrations in the non-diabetic rats. The 4% beta-glucan group showed liver cholesterol concentrations even lower than the non-diabetic rats. Thus, yeast beta-glucans are highly effective in lowering liver cholesterol.

Although viscosity is a characteristic of dietary fiber that is well established to be responsible for cholesterol lowering (186, 287), this clearly is not the explanation for the cholesterol lowering effect of yeast beta-glucans, since no increase in intestinal content viscosity was found. However, some other characteristics of dietary fibers are thought to affect hepatic cholesterol concentration as well. There are three potential mechanisms that may contribute to the hepatic cholesterol lowering effects of yeast beta-glucan.

Yeast or fungal beta-glucans were proposed to lower cholesterol levels by increasing fecal sterol excretion. Cheung discovered that feeding the hamsters straw mushroom, with beta-glucan in its fruiting body and mycelium, can significantly reduce plasma and hepatic cholesterol levels (288). In addition, significantly increased fecal sterol excretion and significant greater cholesterol concentration within fecal sterols were found in the hamster with straw mushrooms consumption, which confirmed that fungal beta-glucan may decrease hepatic cholesterol concentration by increasing cholesterol excretion. In addition, Yoshida et al. found feeding rats the insoluble parts of *Kluyveromyces marxianus* (KM) with enriched cell walls, which is a species of yeast with beta-glucan in its cell walls, can significantly decrease plasma and hepatic cholesterol concentrations and increase fecal

sterol excretion, while the feeding of rats the soluble parts of KM, which has little cell wall, or feeding rats the beta-glucanase treated KM showed no such effects (279). These findings suggest that beta-glucan is the major active component of KM, which had hypocholesterolemic effects by increasing fecal sterol excretion.

Besides the increased fecal sterol excretion with yeast beta-glucan consumption, Yoshida et al. in his study also observed significantly increased cecal short chain fatty acids (SCFA) production, especially significant higher levels of propionate and decreased hepatic cholesterol 7 alpha-hydroxylase (CYP7A) activity, which is an enzyme involving in cholesterol metabolism in liver, in KM fed groups compared to the control group without added KM (279). Yoshida's study suggested that SCFAs, especially propionate, formed from yeast beta-glucan fermentation can inhibiting liver cholesterol synthesis. His finding is consistent with our results that yeast beta-glucan is partially fermented, which will generate SCFAs, primarily acetate, propionate, and butyrate, in rats' large intestine. In addition, Wright et al. discovered that propionate at certain concentration can produced a statistically significant inhibition on cholesterol and fatty acids biosynthesis in rat's liver, and this inhibit effects had a positive relationship with propionate concentration (289). However, the inhibiting effects of propionate on hepatic cholesterol synthesis has not been consistently found. Nishina et al. demonstrated that propionate at certain concentration decreased fatty acids synthesis in hepatocytes, however, propionate did not affect overall sterol synthesis in liver (207), indicating that the propionate did not have significant inhibitive effects in reducing hepatic cholesterol synthesis. Moreover, by comparing the inhibition effects of propionate on cholesterol synthesis between human and rat hepatocytes, Lin et al. proposed that propionate had a dose-related suppressive effect on acetate incorporation into cholesterol in both human and rat hepatocytes, however, a similar does-related inhibitive effect of propionate on hepatic cholesterol synthesis was only observed in rat hepatocytes but not in human hepatocytes (208). Thereby, the effects of propionate, produced from fiber fermentation in colon, on hepatic cholesterol

synthesis is still unclear.

Yeast beta-glucan is its immune-modulatory effect as a binding component to one of the pattern recognition receptors (PRRs), dectin-1 (226). PPRs are a group of proteins that can recognize a diverse collection of microbial pathogens to activate the innate immune response (290). Dectin-1 is expressed on the surface of macrophages and dendritic cells (DCs), which are antigen-presenting cells that activate immune responses (291). Binding of beta-glucan to dectin-1 result had been found to stimulate synthesis and release of anti-inflammatory cytokines, such as interleukin-2 (IL-2), interleukin-10 (IL-10), and tumor growth factor (TGF-β1) productions, thus showing that beta-glucan has a significant effect in inducing innate immune responses (226). The anti-inflammatory cytokines generated from immune responses may contribute to the hypocholesterolemic effects of yeast beta-glucan consumption. IL-2 was first discovered to have a hypocholesterolemic effect in early 1990s when IL-2 was used to treat advanced cancer patients and a profound reduction (up to 64% serum cholesterol reduction) in cholesterol levels was noticed (228). A subsequent study that treated cancer patients with IL-2 observed an acute decrease (64% reduction) in lecithin–cholesterol acyltransferase (LCAT) activity, the enzyme that converts free cholesterol into cholesteryl esters (292). In addition, decreased lipoprotein lipase (LPL) and hepatic lipase (HL) levels were also observed in patients with IL-2 treatment; both LPL and HL are lipases that hydrolyze triglycerides in lipoprotein (292). Patients experienced an average of 52% reduction in cholesterol levels compared to cholesterol levels prior to IL-2 treatment. This result suggested that cytokines, such as IL-2, may decrease cholesterol synthesis by inhibiting the activity or concentration of cholesterol-synthesis-related enzymes, leading to hypocholesterolemia in patients. Thus, there appears to be a plausible mechanism by which beta-glucan could reduce cholesterol via an immune response. However, further experiments are needed to confirm the relationship between the immune cytokines responses from yeast beta-glucan with its cholesterol-lowering effect.

Despite the significant reduction of hepatic cholesterol, there were no

differences in liver lipid concentrations among any of the groups, suggesting that fatty liver was not successfully induced in diabetic animal models in our study. This is consistent with our speculation that, in our study, the animals exhibited more of a type I diabetes than type II diabetes, since NAFLD is not typically found in type I diabetes, as it is in type II diabetes (293). Our diabetic animals were experiencing diabetic wasting, as indicated by their slower rate of growth and their greater urinary glucose excretion, relative to the non-diabetic animals. Normally, after a meal, the high insulin induces FFA and TAG synthesis in the liver and inhibits hepatic beta-oxidation (40). However, with limited insulin secretion in our diabetic animals, there would have been no stimulation of hepatic TAG and FFA synthesis, and hepatic beta-oxidation would have increase due to the lack of glucose uptake by the liver. Consequently, there would have been no accumulation of fat in the liver. Therefore, in our study, due to the severity of diabetes that was induced, we could not evaluate the effect of yeast beta-glucans on the development of fatty liver.

The results of this study indicates that the yeast beta-glucan consumption does not increase small intestinal contents viscosity. However, yeast beta-glucan can be partially fermented. The failure of non-viscous yeast beta-glucans to improve glycemic control in the diabetic rats, whereas viscous cereal-based beta-glucans clearly do, implies that viscosity is likely the important characteristic of beta-glucans responsible for improving hyperglycemia in diabetes rather than fermentability. Yeast beta-glucans did show a significant cholesterol lowering effect in the liver. However, the mechanism for this reduction in hepatic cholesterol is uncertain. Finally, because the animal models had more type I diabetes characteristics than type II diabetes, and fatty liver was not successfully induced in the diabetic animals, we cannot predict whether yeast beta-glucan may slow the development of NAFLD. Thus, we conclude that yeast beta-glucan, which have no viscosity but some fermentability, does not improve glycemic control in diabetes but does reduce hepatic cholesterol.

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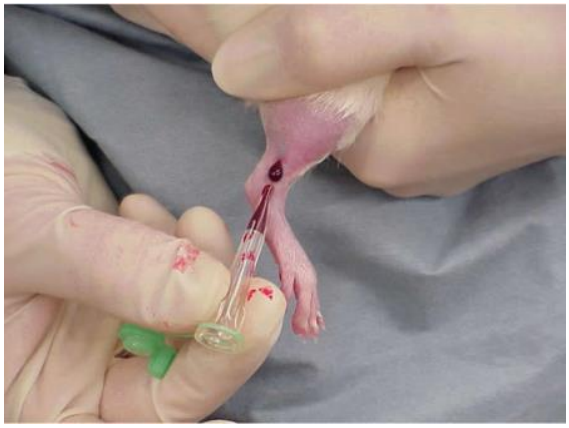
Appendices

Appendix A: Oral glucose tolerance test assay

1. Reagent:
2. 20% D-glucose
3. Dissolve 20 g of D-glucose in 100 mL H2O
4. EDTA solutions
5. Dissolve 300 mg EDTA into 5 mL DI water.
6. Instrument
7. High-speed centrifuge
8. Material
9. Capillary tubes with EDTA
10. Mark each capillary tube with rat number and collection time spot. Pipette 10µl EDTA solution into each tube
11. Procedure
12. Fast animals one night with water supply for about 16 hours the day before experiment.
13. Clean the left leg with 70% ethanol and rub it for better circulation
14. Animals are weighed, collect the fasting blood samples as a baseline
15. Gently extend the thigh and remove hair with clipper
16. 
17. Use a razor blade 105, prick the saphenous vein and withdraw the blade, allowing the blood to flow
18. Collect the blood (0.2 mL) with a micro capillary tube with EDTA in tubes

19. 
20. Give the rat 2 g/kg body weight of 20% D-glucose solution with a syringe intragastrically using a gavage needle (start counting time)
21. At 30, 60, 90 and 120 minutes, blood is sampled from the left leg saphenous vein using a capillary tube with EDTA
22. Blood samples are spun in microfuge at 14,000 rpm for 10 min
23. Transfer serum to a clean tube

Appendix B: Pyruvate tolerance assay

Equipment:

1. AlphaTRAK® blood glucose monitor
2. AlphaTRAK® Blood Glucose TEST STRIPS
3. razor blades 105

Solutions:

1. saline
2. sodium pyruvate solution (dissolved in saline)

Procedures:

1. Weigh the animals (rats or mice) the day before test, get the total weight for all rats.
2. Fast the animals for 16 hours before the pyruvate tolerance test.

3. Make normal saline (0.9% NaCl) as a solvent **(DO NOT USE PHOSPHATE BUFFER!!!!!)** to dissolve the sodium pyruvate.
4. The dose for rats is 1 g pyruvate acid /kg body weight, so the dose for sodium pyruvate is 110 g/mol (sodium pyruvate mole weight) /88 g/mol (pyruvate acid mole weight) = 1.25 g sodium pyruvate /kg body weight
5. Calculate the sodium pyruvate needed for animals and multiple by 1.6 to make sure the pyruvate solution is sufficient
6. Dissolve the sodium pyruvate in saline (0.9% sodium chloride in DI water) and put in cold room
7. Calculate the volume for each rat based on their body weight and prepare the syringe (1 mL for small rat and 3 mL for large rat) and needles (22 gauge × 1 ½ inches) for injection
8. Test the initial blood glucose from the upper tail **(DO NOT USE THE END OF THE TAIL, DATA CANNOT BE TRUSTED!!!!!)** by glucometer.
9. Use needle and syringe to do the intraperitoneal injection on belly, volume is pre-determined by calculation
10. Put the rat back and keep fasting
11. Test the blood glucose levels from upper tail at 30, 60, 90, 120 minutes by glucometer.
12. Give foods back to animals after pyruvate tolerance test.

Warning:

1. Phosphate buffer will cause cell necrosis on skin cells! Phosphate buffer is extremely dangerous above 100 mM in rats and will kill them!

2. The blood glucose levels from the end of the tail on rats cannot be trusted because the blood is less circulating at the end of the tail

Appendix C: Plasma glucose assay

1. Thaw the plasma, gentle vortex the tube after it is thawed.
2. Pipette into the glass tubes 50 µL of either glucose standard (1.75 mg/mL) or plasma in duplicate, as shown below:

Tube	Volume of standard to pipette (µL)	Volume of plasma to pipette (µL)	Volume water to pipette (µL)	Absorbance at 460 nm
A (Reagent Blank)	0	0	50	
A (Reagent Blank)	0	0	50	
B	10	0	40	
B	10	0	40	
C	20	0	30	
C	20	0	30	
D	30	0	20	
D	30	0	20	
E	40	0	10	
E	40	0	10	
F	50	0	0	
F	50	0	0	
Plasma 1	0	25	25	
Plasma 1	0	25	25	
Plasma 2	0	25	25	
…..	0	25	25	

3. Add 2.5 mL of Glucose oxidase-peroxidase (GOPOD) reagent using the repipet. Vortex each tube.
4. Incubate the tubes for 10 minutes in the water bath.
5. Measure absorbance of the solution in each tube at 460 nm using the spectrophotometer.

Reagents:

Stock glucose standard: (3.5 mg/mL) 0.35 g anhydrous primary standard dextrose in 100 mL dd H_2O. Add 0.1 g benzoic acid/ dL for preservation.

GOPOD: Glucose oxidase-peroxidase reagent 50 mL

30 mg glucose oxidase (Goal is 285 units/ 50 mL)

15 mg peroxidase (Goal is 1200 units/ 50 mL)

45 mg reduced sodium p-diphenylaminesulfonate

50 mL 0.1 M citrate buffer, pH=5.5. Use **sodium citrate**!

Appendix D：Glycated hemoglobin assay

Reagents:

1. GLYCO-Tek Affinity Columns Ingredients: Each column contains cellulose resin covalently bonded to dihydroxyboryl groups, in a low ionic strength preservative solution containing 0.1% sodium azide. (stored in the dark at 15 to 30 °C prior to use)
2. GLYCO-Tek Hemolysate Reagent Ingredients: The reagent contains 0.05 M magnesium chloride, 2% Triton X-100, 0.1 M glycine and sodium azide as a preservative. (stored at 2 to 6 °C prior to use).
3. GLYCO-Tek Developer A Ingredients: The reagent contains 0.05 M magnesium chloride, 0.1 M glycine, and sodium azide as a preservative; pH 8.1-8.6 (stored at room temperature before using).
4. GLYCO-Tek Developer B Ingredients: The reagent contains sorbitol, buffer and 0.1% sodium azide as a preservative; pH 6.0 (stored in the dark at 2 to 6 °C prior to use).

Equipment:

Standard spectrophotometer

Procedure:

1. Obtain a small collection tube and a large collection tube and a GLYCO-Tek affinity column for each animal and control to be tested.
2. Preparation of the animal sample(s)
   a. Place 50 µL of packed cells or whole blood in a small disposable glass test tube.

   b. Add 400 µL GLYCO-Tek Hemolysate Reagent to the test tube.

   c. Vortex the tube to ensure complete hemolysis of the sample. Excessive foaming should be avoided.

   d. Allow the sample(s) to stand at least 5 minutes but not more than 45 minutes prior to use.
3. Prepare the GLYCO-Tek Affinity Column

   a. Up-end each column twice to remove resin adhering to the top cap closure. Place the column in the Quik Column Rack.

   b. Remove the top cap closure and resuspend the resin completely using a

disposable Pasteur pipette. Suck the resin in and out of the pipette as moving it down to the top of the filter in the column.

c. Place the columns over a container and remove the bottom tip closure. Allow solution to elute and the resin to pack.

d. When the resin has settled to a level of supernatant has drained to below the shoulder of the column, remove the liquid to the top of the resin bed with a transfer pipette.

e. Add 3 mL Developer A to each column, and allow complete elution of the developer into the sink or a container.

4. After the developer has drained from the column, place the column over a Large collection tube (non-GHb). Do not allow the column to set dry for longer than 10 minutes.
5. Apply 50 µL of sample hemolysate prepared from packed cells, or 100 µL of hemolysate prepared form whole blood to the top of the resin bed.
6. Allow the sample to set on the resin for 8 minutes (acceptable range 6-10 minutes).
7. After 8 minutes' incubation, add 0.5 mL GLYCO-Tek Developer A to each column, washing any hemolysate adhering to the sides of the column into the resin. Allow to elute.
8. Apply an additional 4 mL GLYCO-Tek Developer A to the column carefully avoiding any disturbance of the resin bed. Allow complete buffer elution. The eluent in the Large Collection Tube contains the non-glycated hemoglobins.
9. Adjust the volume in the Large Collection Tube to 15 mL with deionized water.
10. Place the column over a Small Collection Tube and carefully add 3.0 mL GLYCO-Tek Developer B to the column avoiding any disturbance of the resin bed.
11. Allow complete buffer elution into the Small Collection Tube (GHb). The eluent in the small tube contains all the glycated hemoglobins.
12. Invert the Large and Small Collection Tubes in such a manner that the air bubble travels completely from top to bottom of the tube and back to the

top. Repeat two times for a total of three inversions.

13. Transfer collected fraction to cuvette and read cuvette immediately after last inversion.
14. Use a standard spectrophotometer at wavelength to 415 nm to read each sample including the control

Calculation of the results:

results are calculated by the following formula:

$$\frac{\text{Abs.of GHb Tube x 100\%}}{\text{(Abs.of GHb Tube) + 5.0 (Abs.of non-GHb Tube)}} = \%\ \text{GHb}$$

%GHb = percentage of glycated hemoglobins in the sample

Abs.of GHb tube = absorbance of the contents of the small collection tube at a wavelength of 415 nm

Abs. of non-GHb tube = absorbance of the contents of the large collection tube at a wavelength of 415 nm.

5.0 = dilution factor (15 mL or non-GHb tube/3 mL of GHb tube)

100 = percentage conversion factor

Appendix E: Liver lipid extraction assay

Procedures:

1) Weigh out ~1.00 gram of liver tissue and transfer to homogenizing tube.

2) Add 10 mL Chloroform:MeOH (2:1).

3) Homogenize for ~15-20 seconds.

4) Filter homogenate through a #1 Whatman filter paper into a glass screw top tube.

5) Rinse homogenizer with 15 mL Chloroform:MeOH (2:1) and pick out any tissue trapped on probe.

6) Pour rinse through filter paper into tube.

7) Wash filter paper with an additional 5 mL Chloroform:MeOH (2:1).

8) Add 6 mL 0.9% NaCl to filtrate. This represents a volume of 0.2 times the volume of Chloroform:MeOH used.

9) Vortex to mix aqueous and nonaqueous phases.

10) Centrifuge for 1 minutes at 750 RPM to separate phases. Alternatively, let the tubes stand until phases separate.

11) Remove aqueous upper phase with a transfer pipet and discard.

12) Gently layer 3 mL of MeOH:H2O (1:1). Rinse sides of tubes while adding the MeOH:H2O.

13) Remove this upper phase with a transfer pipet and discard.

14) Transfer the extracted lipid in Chloroform:MeOH (2:1) and transfer rinse to lipid vial (weigh each vial before transfer).

15) Evaporate Chloroform under N2. (Gravimetric Step)

16) Weigh the vial after evaporation.

17) Calculate the weight of lipid extraction by subtracting the vial weight before evaporation from the vial weight after evaporation.

Appendix F: Enzymatic cholesterol assay

Reagents:

1) 50 mM Sodium Phosphate, Dibasic (MW = 141.96). Make 1 L, pH=6.9 (7.1 g/L).

2) Stock Phenol Reagent: 500 mL

Dissolve 3.8 g Phenol crystals in 400 mL Phosphate Buffer. Adjust volume to 500 mL with Phosphate Buffer. Store at 4℃ (up to 1 month).

3) Stock Mixed Reagent: 500 mL

Dissolve in 400 mL Phosphate Buffer:

0.203 g 4-aminoantipyrine

1.292 g Sodium Cholate

7.46 g Potassium Chloride

1.0 mL Triton X-100

Adjust volume to 500 mL with Phosphate Buffer. Store at 4 ˚ C (up to 1 month).

4) Working Reagent: (shelf life: 3-4 days)

	Amt (UI)/30 mL	Amt (UI)/100 mL	Amt (UI)/200 mL
Reagent	(mL)	(mL)	(mL)
Stock Mixed Reagent	15	50	100
Stock Phenol	15	50	100
Cholesterol Oxidase (UI)	7.5	25	50
Cholesterol Esterase (UI)	7.5	25	50
Peroxidase (UI)	375	1250	2500

Store in ice bath until ready to use.

****NOTE**** For free cholesterol assay, Omit Esterase

A) Standard Curve:

Use 1 mg/mL concentration of cholesterol in Chloroform:MeOH (2:1). Use 0, 5, 10, 15, 20, and 25 µL concentrations (depends on cholesterol in sample).

Add 50 µL Triton X-100/Acetone to each tube, Vortex.

Dry under N2 gas. (Triton Sol'n = 1.5 g Triton in 10 mL Acetone)

B) Samples: Reconstitute the liver fat with a set volume for all samples (10 mL) in Chloroform:MeOH (2:1).

Total Cholesterol: 10 µL assayed (from 10 mL liver lipid extract)

Add 50 µL Triton X-100/Acetone.

Dry solvent under N2 gas.

C) Assay: Add 100 µL dH2O to each tube, Vortex.

Add 1 mL Working Reagent/sample, Vortex.

Incubate at 37 ºC for 10 min. Cool to room temperature.

Read absorbance at 500 nm.

Appendix G: Small intestine content viscosity measurement assay

Procedures:

1. Excise the small intestines from the end of gastro through the cecum during the dissection.

2. Collect the intestinal contents into tubes by finger stripping, and store the tube on ice.

3. Centrifuge the tubes with small intestine contents @ 30,000 *g* for 30 minutes @ 37 ºC.

4. Transfer the supernatants to new tubes.

5. Transfer 0.5 mL supernatant from each sample into Wells-Brookfield cone

6. Measure the standard silicone on the dial on a cone/plate viscometer (model RVT, Brookfield Engineering, Stoughton, MA).

7. Measure the supernatants from each sample @ 37 ºC at all possible shear rates between 1.15 and 230 s^{-1} within 6-7 hr of collection.

8. Plot the viscosity values versus shear rate on a log-log scale.

9. Viscosity is estimated by extrapolation of the regression line to a shear rate of 23.0 s^{-1}.

Note: Viscosities are expressed as millipascal seconds (mPa·s).

Appendix H: Yeast beta-glucan assay

Assay Kit: Mushroom and Yeast Beta-glucan Assay Procedure. (Megazymes Inc., Ireland)

Kits contain:

Bottle 1: exo-1,3-β-Glucanase (100 U/mL) plus β-Glucosidase (20 U/mL) ammonium sulphate suspension, 2.0 mL. Stable for > 4 years at 4 °C.

Bottle 2: Amyloglucosidase (1,630 U/mL) plus invertase (500 U/mL) solution in 50 % (v/v) glycerol, 20 mL. Stable for ~ 2 years at 4°C or > 4 years at -20 °C. **Bottle 3:** GOPOD Reagent Buffer. Buffer (50 mL, pH 7.4). p-

hydroxybenzoic acid and sodium azide (0.095%). Stable for > 4 years at 4 °C.

Bottle 4: GOPOD Reagent Enzymes. Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder. Stable for > 5 years at -20 °C.

Bottle 5: D-Glucose standard solution (5 mL, 1.00 mg/mL) in 0.2% (w/v) benzoic acid. Stable for > 5 years at room temperature. Bottle 6: Control yeast β-glucan preparation (~ 2 g, β-glucan content stated on the bottle label). Stable for > 5 years at room temperature.

Reagent:

1. Sodium acetate buffer (200 mM, pH 5.0). Add 11.6 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water and adjust to pH 5.0 using 4 M (16 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 L. Stable for ~ 1 year at 4 °C.
2. Sodium acetate buffer (1.2 M, pH 3.8). Add 69.6 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water and adjust to pH 3.8 using 4 M sodium hydroxide. Adjust the volume to 1 L with distilled water. Stable for > 2 years at room temperature.
3. Potassium Hydroxide (10 M). In a well ventilated fume cupboard, add 561 g of KOH to 700 mL of distilled water and dissolve by stirring. Allow the solution to cool to room temperature and then adjust the volume to 1 L. Stable for > 2 years at room temperature.
4. Potassium Hydroxide (2 M). Add 112 g of KOH to 800 mL of distilled water and dissolve by stirring. Adjust the volume to 1 L. Stable for > 2 years at room temperature.
5. Sulfuric acid (12 M, 72% w/w). In a well ventilated fume cupboard, carefully add 640 mL of concentrated acid (98%, sp. gr. 1.835) to 300 mL of distilled water. Dilute to 1 L and mix well. Stable at room temperature for > 4 years.

Equipment:

Bench centrifuge, Spectrophotometer, and Vortex mixer.

Preparation of the reagent:

1. Add 9 mL of 200 mM sodium acetate buffer (pH 5.0) to bottle 1. Divide into appropriately sized aliquots and store in polypropylene tubes at -20 °C between use and on ice during use. Once diluted, the reagent is stable for > 2 years at -20 °C.
2. Dilute the contents of bottle 3 to 1 L with distilled. This is Solution 1. Use immediately.
3. Dissolve the contents of bottle 4 in approx. 20 mL of solution 1 and quantitatively transfer to the bottle containing the remainder of solution 1. Cover this bottle with aluminum foil to protect the enclosed reagent from light. This is Glucose Determination Reagent (GOPOD Reagent). Stable for ~ 3 months at 2-5 °C or > 12 months at -20 °C. If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots. Do not freeze/thaw more than once. The absorbance of this solution should be less than 0.05 when read against distilled water.

Procedures:

A. Measurement of Total Glucan (α-glucan + β-glucan) plus D-Glucose in Oligosaccharides, Sucrose and free D-Glucose

a. Solubilization and partial hydrolysis of total glucan (α-glucan + β-glucan) plus D-glucose in oligosaccharides, sucrose and free D-glucose

1. Mill the sample to pass a 1.0 mm screen using a Retsch centrifugal mill, or similar.
2. Add milled sample [approx. 90 mg, weighed accurately] to a 20 x 125 mm Fisher Brand culture tube. Tap the tube to ensure that all of the sample falls to the bottom of the tube.
3. Add 2.0 mL of ice cold 12 M sulphuric acid to each tube, cap the tubes and stir them vigorously on a vortex mixer. Place the tubes in an ice-water

bath and leave them there for 2 h. Over this period of time, vigorously stir the tube contents (for 10-15 sec) several times on a vortex mixer (to ensure complete dissolution of the β-glucan).

4. Add 4 mL of water to each tube, cap the tubes and vigorously stir the contents on a vortex mixer for 10 sec. Then add 6 mL of water, cap the tubes and stir the contents for a further 10 sec.
5. Loosen the caps on the tubes and place them in a boiling water bath (~ 100°C). After 5 min, tighten the caps and continue the incubation for 2 h.
6. Cool the tubes to room temperature and carefully loosen the caps.
7. Quantitatively transfer the contents of each tube to a 100 mL volumetric flask using a wash bottle containing 200 mM sodium acetate buffer (pH 5).
8. Add 6 mL of 10 M KOH solution to the volumetric flask and adjust to volume with 200 mM sodium acetate buffer (pH 5). Mix the contents well by inversion and collect an aliquot of the sample in a polypropylene centrifuge tube.
9. Centrifuge an aliquot of the solution at 1,500 *g* for 10 min.

b. Measurement of total glucan plus D-glucose in sucrose and free D-glucose.

10. Transfer 0.1 mL aliquots (in duplicate) of filtered or centrifuged extract to the bottom of glass test tubes (16 x 100 mm).
11. Add 0.1 mL of a mixture of exo-1,3-β-glucanase (20 U/mL) plus β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 5.0) to the bottom of each tube, mix the tube contents on a vortex mixer and incubate at 40 °C for 60 min.
12. Add 3.0 mL of GOPOD Reagent to each tube and incubate at 40 °C for 20 min.

13. Measure the absorbance of all solutions at 510 nm against the reagent blank.

Note:

With each set of determinations, include at least one control yeast or mushroom preparation. Also include reagent blanks and glucose standards of 100 µg (in quadruplicate). Run these through the entire incubation procedure with GOPOD Reagent

The **reagent blank** consists of 0.2 mL of sodium acetate buffer (200 mM, pH 5.0) + 3.0 mL GOPOD Reagent.

The **D-glucose standard** consists of 0.1 mL D-glucose standard (1 mg/mL) + 0.1 mL of sodium acetate buffer (200 mM, pH 5.0) + 3.0 mL GOPOD Reagent.

B. Measurement of α-Glucan (phytoglycogen and starch) plus D-glucose in sucrose and free D-glucose.

a. Solubilization, hydrolysis and measurement of α-glucan, D-glucose from sucrose and free D-glucose

1. Add milled sample (approx. 100 mg, weighed accurately) to a 20 x 125 mm Fisher Brand culture tube. Tap the tube to ensure that all of the sample falls to the bottom of the tube.
2. Add a magnetic stirrer bar (5 x 15 mm) followed by 2 mL of 2 M KOH to each tube and suspend the pellets (and dissolve the phytoglycogen/starch) by stirring for approx. 20 min in an ice/ water bath over a magnetic stirrer.
3. Add 8 mL of 1.2 M sodium acetate buffer (pH 3.8) to each tube with stirring. Immediately add 0.2 mL of amyloglucosidase (1,630 U/mL) plus invertase (500 U/mL), mix well and place the tubes in a water bath at 40 °C.
4. Incubate the tubes at 40 °C for 30 min with intermittent mixing on a vortex stirrer.

5. **For samples containing > 10% α-glucan content**; quantitatively transfer the contents of the tube to a 100 mL volumetric flask (using a water wash bottle) and adjust to volume with water. Mix well. Centrifuge an aliquot of the solution at 1,500 *g* for 10 min or filter through Whatman No. 1 filter paper (9 cm).
6. **For samples containing < 10% α-glucan content**; directly centrifuge the tubes at 1,500 *g* for 10 min (no dilution). For such samples the final volume in the tube is approx. 10.3 mL. In some cases, an appropriate allowance for volume should be made in the calculations.
7. Transfer 0.1 mL aliquots (in duplicate) of either the diluted or undiluted supernatants into glass test tubes (16 x 100 mm), add 0.1 mL of sodium acetate buffer (200 mM, pH 5.0) plus 3.0 mL of GOPOD reagent and incubate at 40 °C for 20 min.
8. Measure the absorbance of all solutions at 510 nm against the reagent blank.

NOTE: Mushroom and yeast samples generally contain < 10% α-glucan. However, some commercial mushroom mycelia are grown on cereal grains, and in this case, the starch content of the recovered product can be as high as 75% w/w. This method is **NOT** applicable to the analysis of yeast β-glucan in the presence of cellulose (1,4-β-D-glucan).

Calculation

Total Glucan (% w/w) (+ oligomers etc.) = ΔE x F x $\frac{100}{0.1}$ x $\frac{1}{1000}$ x $\frac{100}{W}$ x $\frac{162}{180}$

= ΔE x F/W x 90

α-Glucan (% w/w) (+ oligomers etc.) = ΔE x F x 1000 (or 103) x $\frac{1}{1000}$ x $\frac{100}{W}$ x $\frac{162}{180}$ = ΔE x F/W x 90 (final volume 100 mL) = ΔE x F/W x 9.27 (final volume 10.3 mL)

b-Glucan = Total Glucan (+ oligomers etc.) - a-Glucan (+ oligomers etc.)

where:

ΔE = reaction absorbance – blank absorbance.

F = a factor to convert absorbance to μg of D-glucose = 100 (μg of the D-glucose standard)

GOPOD absorbance for 100μg of D-glucose standard.

W = weight of sample analyzed.