THE EFFECTS OF MANGO AND ITS COMBINATION WITH ROSIGLITAZONE ON CLINICAL PARAMETERS IN MICE FED HIGH FAT DIET

By

SIN EE TER

Bachelor of Science in Dietetics

Iowa State University

Ames, Iowa

2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2012

THE EFFECTS OF MANGO AND ITS COMBINATION WITH ROSIGLITAZONE ON CLINICAL PARAMETERS IN MICE FED HIGH FAT DIET

Thesis Approved:

Dr. Edralin A. Lucas

Thesis Adviser

Dr. Barbara Stoecker

Dr. Brenda J. Smith

Dr. Sheryl A. Tucker

Dean of the Graduate College

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Obesity has become a major health problem worldwide. Its prevalence has doubled the last two decades [1]. An estimated 200 million men and nearly 300 million women were obese in the year 2008 [2]. According to the National Health and Nutrition Examination Survey (NHANES), in the United States alone, more than ⅓ or 35.7% of the adult population (20 years and above) and 16.9% of the children and adolescents (2 – 19 years old) were obese in 2009 - 2010 [3]. This represents 40.6 and 37.5 million of adult women and men, respectively, and 5.5 and 7.0 million girls and boys, respectively.

The World Health Organization (WHO) defines obesity as a disorder when excessive fat is accumulating in the body, such that it may bring adverse health effects [2]. Obesity is mainly caused by imbalance of energy intake, where more energy is consumed than is expended [4]. Diets high in calories and fat content have been shown to increase body weight and body fatness [5].

Body mass index (BMI) is a commonly used screening tool to assess obesity in a population. It is calculated as weight in kilograms divided by height in meters squared. Individuals with BMI of 18.5 - 24.9 kg/m^2, 25 - 30 kg/m^2, and >30 kg/m^2 are categorized as normal, overweight, and obese, respectively. Although BMI does not directly measure body

fatness, it is a relatively reliable indicator of body fatness for most people, as BMI correlates to direct measures (i.e. underwater weighing and dual energy x-ray absorptiometry (DXA)) of body fat [4]. High BMI is also associated with obesity-related morbidities, such as high blood pressure and high blood cholesterol level [6], indicating its relevance as a measure of body fatness. Besides BMI, waist circumference can also be used to assess obesity. A waist circumference greater than 40 inches in men and 35 inches in women indicates central adiposity, and it is one of the indicators for metabolic syndrome [7].

Alongside the increased prevalence of obesity is an increased incidence of many chronic conditions. Obesity is shown to be a risk factor to many chronic diseases, such as stroke, coronary heart disease, gallbladder disease, osteoarthritis, certain types of cancer, hypertension, type 2 diabetes mellitus, and dyslipidemia [4]. The relationship between obesity and type 2 diabetes is especially strong. Obese individuals were found to be 1.65 times more likely than normal weight individuals to develop type 2 diabetes [8].

Because of the increased prevalence of obesity, the number of cases of type 2 diabetes has also increased. The Centers for Disease Control and Prevention (CDC) defines diabetes as a group of diseases marked by high blood glucose levels due to impaired insulin production and/or impaired insulin action to signal cells to uptake glucose [9]. Worldwide, more than 346 million people have diabetes [10]. In the United States, diabetes is affecting 25.8 million individuals or 8.3% of the population [11]. Research showed that diabetics had 50% greater mortality risk than people without diabetes of similar age. The estimated direct and indirect costs for diabetes were $176 billion in 2007 [11].

Due to the prevalence and severity of diabetes, pharmaceutical companies have developed numerous drugs to lower blood glucose level. There are six classes of oral medications for diabetes that are sold in the United States: biguanides, thiazolidinediones, sulfonylureas,

alpha-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors (DPP4-inhibitors), and meglitinides [12]. They work through different mechanisms in lowering blood glucose levels in diabetics.

Biguanides, such as metformin are suggested to reduce hepatic glucose output, increasing insulin action and insulin-mediated glucose utilization in peripheral tissues [12, 13]. However, based on the review by Lorenzati *et al.*[13], the use of metformin is associated with some side effects, including metallic taste, mild anorexia, nausea, and diarrhea. Sulfonylureas, on the other hand, are proposed to work by stimulating insulin release from pancreatic β-cells [12], and to a lesser extent by improving insulin resistance in peripheral tissues [13]. Side effects associated with sulfonylurea use include hypoglycemia and weight gain [13]. Alpha-glucosidase inhibitors inhibit the breakdown and absorption of carbohydrates [12], but are associated with flatulence, abdominal discomfort, bloating, and diarrhea [13]. In contrast, DPP4-inhibitors act by stimulating insulin secretion, inhibiting glucagon secretion, and possibly preserving β-cell mass and inhibiting apoptosis of β-cell [13]. DPP4-inhibitors are associated with increased risk for infection, headache, back pain, and osteoarthritis. Alternatively, meglitinides stimulate insulin release from pancreatic β-cells [12], but are associated with hypoglycemia [13]. Thiazolidinediones (TZDs), such as rosiglitazone, increase insulin sensitivity [12, 14] by increasing glucose uptake and utilization by muscle and adipose tissues, and inhibiting hepatic gluconeogenesis [13]. However, the use of rosiglitazone is associated with some side effects including edema [15-18], weight gain [15, 16, 19], fractures [20-22], congestive heart failure [15, 23, 24], myocardial infarction [25, 26], and increased risk of death from cardiovascular causes [25].

Because of the deleterious side effects associated with rosiglitazone and other pharmacological agents, natural food products are being investigated for their possible beneficial effect in lowering blood glucose level. Fruits, like mangos, were demonstrated to have glucose-

lowering effects [27-39] possibly due to fiber, vitamins, minerals and other bioactive compounds [40]. Most of the studies investigating the glucose-lowering properties of mango were done using its extract especially from the stem bark, which is rich in mangiferin. Mangiferin, a phenolic compound found in mango [41], when administered orally to streptozotocin (STZ)-induced diabetic rats was shown to significantly decrease blood glucose level [33].

Only a few studies have been done to investigate the health benefits of the mango fruit (pulp). Perpetua and Salgado [38] found that alloxan-induced diabetic rats when fed diet containing 5%, 10%, and 15% (by weight) of mango flour for 30 days had significantly lower blood glucose levels compared to the control group. An earlier study by Lucas and colleagues [39] compared the glucose-lowering properties of two doses of freeze-dried mango pulp powder (1% or 10%) to that of rosiglitazone in mice fed high fat diet. They found that mango supplementation was able to reduce blood glucose and improve lipid profiles in mice fed high fat diet.

The *aim* of this study is to investigate the effectiveness of mango (1% or 10% by weight) in combination with a lower dose of rosiglitazone (25 ppm) compared to a higher dose of rosiglitazone (50 ppm) alone in lowering blood glucose concentrations and modulating body composition and other clinical parameters in mice fed high fat diet. Since rosiglitazone was shown to be associated with some side effects, a lower dose of rosiglitazone could potentially reduce or delay the side effects associated with the use of higher doses of rosiglitazone. Our *null hypothesis* is that a lower dose of rosiglitazone (25 ppm) when combined with mango will not be as effective as rosiglitazone alone (50 ppm) in lowering blood glucose level and modulating body composition and other clinical parameters in mice fed high fat diet. The present study also sought to confirm the earlier findings by Lucas and colleagues [39] on the effectiveness of freeze-dried mango pulp powder (1% and 10%) in lowering blood glucose concentrations compared to the drug rosiglitazone in mice fed high fat diet.

CHAPTER II

LITERATURE REVIEW

Type 2 diabetes

Prevalence and cost

Worldwide, more than 346 million people have diabetes [10]. In the United States (U.S.) alone, diabetes is affecting 25.8 million people or 8.3% of the population [11]. In the year 2010, about 1.9 million and 79 million Americans were newly diagnosed with diabetes and prediabetes, respectively. Prediabetes is a condition when the fasting plasma glucose or hemoglobin A1c levels (HbA1c) of an individual are between 100 - 125 mg/dL or 5.7% - 6.4%, respectively. People diagnosed with prediabetes have an increased risk of developing type 2 diabetes. Additionally, gestational diabetes cases were reported to be 2% - 10% of all pregnancies in the U.S. Research showed that women that had gestational diabetes have a 35% - 60% chance of developing diabetes in the next 10 - 20 years [11].

The mortality rate and medical cost due to diabetes is also increasing. Diabetes is the seventh leading cause of death, and was responsible for 71,382 deaths in the U.S. in 2007 [11]. Diabetics were found to have 50% greater mortality risk than non-diabetics of similar age. The estimated total direct and indirect medical cost for diabetes was $174 billion in 2007 [11]. The projected diabetes related medical cost for the year 2032 is $336 billion [42]. Moreover,

according to CDC, individuals diagnosed with diabetes have to spend twice as much on medical expenses as individuals without diabetes.

In addition to high prevalence and cost, diabetes also leads to detrimental health complications. There are two primary types of diabetes-related complications: macrovascular and microvascular complications. Macrovascular complications are large-vessel diseases, including coronary heart disease and stroke [43]. On the other hand, microvascular complications are diseases affecting the small vessels, including diabetic retinopathy, nephropathy, and neuropathy.

Both macro- and microvascular complications increase the risk of death and hospitalization in diabetes [44]. Young and colleagues [44] reported that the hazard ratio of mortality risk associated with two diabetic complications was 1.90, three complications was 2.66, four complications was 3.41, and five or more complications was 7.18. On the other hand, the hazard ratio of hospitalization risk associated with diabetic complications was 1.48 for one complication, 2.27 for two complications, 3.12 for three complications, 4.13 for four complications, and 6.56 for five or more complications [44].

Diabetes is also associated with 2- to 4-fold increased risk of cardiovascular diseases (CVD) when compared to non-diabetic individuals [45]. In 2004, 68% of heart disease deaths and 16% of stroke deaths reported among people aged 65 years or older were related to diabetes [11]. Also, CDC reports that diabetic adults have 2 to 4 times higher heart disease mortality rates and 2 to 4 times higher risk for stroke than non-diabetic adults. The risk of coronary artery disease increases even further with poorly controlled diabetes because insulin resistance can accelerate atherogenesis. Good glucose control: (1) HbA1c level <7%, (2) fasting plasma glucose <100 mg/dL, and (3) non-fasting glucose level <140 mg/dL as recommended by the American Diabetic Association, as well as good lipid control: (1) low-density lipoprotein cholesterol (LDL-C) <100 mg/dL, (2) triglycerides (TG) <150 mg/dL, and (3) high-density lipoprotein cholesterol (HDL-C)

>50 mg/dL for women and >40 mg/dL for men, as recommended by the National Cholesterol Education Panel, can reduce the incidence of CVD.

Aside from getting macrovascular complications, diabetics are also prone to microvascular complications, such as diabetic retinopathy, nephropathy, and neuropathy. Diabetic retinopathy is defined as damage to the eye's retina that occurs with chronic diabetes [46]. It is characterized by signs or symptoms of retinal ischemia and increased retinal vascular permeability [47]. Diabetic retinopathy is the most common microvascular complication in diabetes [48], and the leading cause of blindness in the U.S. [47]. Every year, diabetic retinopathy results in nearly 10,000 new cases of blindness [48]. In 2005 - 2008, 25.8% or 4.2 million diabetics aged 40 years or older had diabetic retinopathy [11], and it is projected to increase to 16 million in 2050 [49]. The direct medical cost of diabetic retinopathy was approximately $500 million among American adults (≥40 years old) in 2004 [49]. The risk of developing diabetic retinopathy depends on the severity of hyperglycemia and the presence of hypertension. Adiga and colleagues [50] found that patients with diabetic retinopathy had the highest blood glucose and HbA1c levels among the other diabetic complications (i.e. foot ulcers, nephropathy, and urinary tract infection). The authors suggested that these results imply that poorer glucose control increases the risk of developing diabetic retinopathy.

Diabetic nephropathy is kidney disease or damage due to diabetes [51], and is characterized by the presence of microalbuminuria (≥300 mg/24 h) [52]. It is the leading cause of renal failure in the U.S. [48], affecting 30 - 50% of all diabetics [53], and accounted for 44% of all new cases of kidney failure in 2008 [11]. In 2008, an estimated 48,374 diabetics had to begin treatment for end stage kidney disease, and a total of 202,290 diabetics with end stage kidney disease were living on chronic dialysis or with a kidney transplant, indicating the severity and prevalence of this complication.

On the other hand, diabetic neuropathy is damage to the nervous system due to hyperglycemia in diabetes [54]. Approximately, 60 - 70% diabetics had mild to severe forms of nervous system damage [11]. Almost 30% of diabetics aged 40 years or older had impaired sensation in the feet, and the severe forms of diabetic neuropathy are a major contributing cause of lower-extremity amputations. In 2006, about 65,700 lower-extremity amputations were performed in diabetics; inferring diabetic neuropathy causes detrimental impact on diabetics.

The statistics discussed above show that diabetes-related macro- and microvascular complications are severe, and they are the common cause of deaths in diabetic patients. Due to their association with the severity and duration of diabetes, good glucose control is crucial to prevent or delay these detrimental complications. Hence, treatments that can help diabetics to maintain good glucose control or improve hyperglycemia will be beneficial in delaying or preventing the development of diabetes associated complications.

Pathophysiology of type 2 diabetes

CDC defines diabetes as a group of diseases marked by high blood glucose levels, which result in impaired insulin production and/or insulin action [9]. Insulin from the pancreatic β-cells helps to modulate blood glucose during the fed state. According to the National Institutes of Health (NIH), diabetes is diagnosed when the fasting blood glucose level of an individual is above 126 mg/dL on two occasions [55]. Aside from fasting blood glucose as a diagnostic tool for diabetes, the American Diabetes Association (ADA) also recommended the blood glycated hemoglobin A1c (HbA1c) test as the alternate diagnostic test for diabetes. The HbA1c test monitors long-term blood glucose control while fasting blood glucose indicates current glucose control. Individuals with a HbA1c concentration greater than 6.5% are diagnosed as diabetes. The combination of these two diagnostic methods is a strong indicator of glucose control in diabetes.

Type 2 diabetes mellitus is a disease manifested by impaired insulin secretion, insulin resistance, and increased hepatic glucose production due to increased glycogenolysis and gluconeogenesis [56]. Under normal condition, glucose level increases following a meal, and this stimulates insulin release from the pancreatic β-cells. Insulin binds to cell surface receptors (insulin receptor) and causes phosphorylation of the β-subunit of the receptor, resulting in an increase in insulin receptor tyrosine kinase activity. Increase in tyrosine kinase activity enhances the phosphorylation of various endogenous protein substrates (e.g. insulin receptor), resulting in a cascading sequence of reactions responsible for the synthesis of RNA, DNA, protein, and intracellular enzymes that affect glucose metabolism. In the fed state, insulin enhances glucose uptake by the peripheral tissues and suppresses hepatic glucose output from glycogenolysis and gluconeogenesis. During fasting, the inhibitory effect of insulin is removed and hepatic glucose production via glycogenolysis and gluconeogenesis is enhanced.

The normal regulation of glucose is disrupted in type 2 diabetes. In type 2 diabetes, insulin-mediated glucose disposal by muscle is reduced, a result of impaired muscle glucose uptake [57]. In addition, glucose production by liver is increased despite elevated insulin level [56]. This phenomenon of hyperglycemia and hyperinsulinemia exemplifies insulin resistance in both muscular and hepatic tissues [57]. The progression of insulin resistance results in β-cell deterioration and impaired insulin secretion and release in addition to continuous hepatic glucose production [56]. Glycogen synthesis, glucose oxidation, and tissue glucose uptake are all impaired in type 2 diabetes.

As a part of impaired glucose metabolism, glucose transport is also impaired in type 2 diabetes. The glucose transporter, GLUT-4, is abundant in adipose cells, skeletal and cardiac muscles [56]. Under normal circumstances, insulin stimulates GLUT-4 translocation to the plasma membrane, resulting in glucose transport across the plasma membrane, a rate-limiting step for glucose metabolism [58]. In type 2 diabetes however, this translocation of GLUT-4

transporters to the plasma membrane is impaired. As a consequence, there is a decreased uptake of glucose into the cell and resistance to insulin-stimulated glycogen synthesis in the muscle, resulting in an accumulation of glucose in the blood, and hence hyperglycemia.

In addition to glucose, lipid metabolism is also affected by diabetes. Increased release of free fatty acids from adipose cells due to enhanced lipolysis contributes to insulin resistance [59]. Elevated serum free fatty acid increases hepatic gluconeogenesis [60] through the production of acetyl-CoA derived from free fatty acid oxidation [61]. In the skeletal muscle, elevated free fatty acids impair glucose metabolism by competing with glucose as energy substrates through the Randle cycle [62]. Long-term exposure of β-cells to elevated free fatty acids also impairs the insulin secretory response to glucose [60].

Contributors to the development of type 2 diabetes

Dietary fat intake

Dietary fat intake is shown to be a risk factor for the development of type 2 diabetes [63]. Thanopoulou and colleagues [63] reported that individuals with recently diagnosed diabetes and with fasting hyperglycemia had higher intake of total dietary fat (especially animal fat) and higher fat percent contribution to the energy intake compared to control even after the adjustment for all contributing variables. Another study conducted by Marshall and colleagues [64] found that fat consumption significantly predicted type 2 diabetes risk in individuals with impaired glucose tolerance. They reported that a 40 g increase in fat intake per day was associated with 3.4-fold increase in the risk of type 2 diabetes after adjusting for contributing variables. Furthermore, trans fatty acid intake was also positively associated with the risk of diabetes in age- and BMI-adjusted analyses [65]. These findings show that total dietary fat intake could lead to and be used as a predictor for future type 2 diabetes incidence.

Obesity

Obesity is also found to be a risk factor for diabetes. The World Health Organization defines obesity as a disorder when excessive fat is accumulating in the body, such that it may bring adverse health effects. Body mass index (BMI) is a commonly used screening tool to assess obesity in a population. It is calculated as weight in kilograms divided by height in meters squared. Individuals with BMI of 18.5 - 24.9 kg/m^2 are categorized as normal, BMI of 25 kg/m^2 and above are overweight, and BMI of 30 kg/m^2 or greater are obese. Although BMI does not directly measure body fatness, it is a relatively reliable indicator of body fatness for most people, as it is correlated to direct measures (i.e. underwater weighing and dual energy x-ray absorptiometry (DXA)) of body fat [4]. The CDC reported that 86.3% and 84.2% obese men and women, respectively, also had diabetes [66]

Mokdad *et al*. [67] found that increased BMI was associated with increased diabetes risk. They reported that obese adults with BMI of 40 or greater were significantly associated with diabetes with odds ratio of 7.37 when compared to normal weight adults. Bray *et al.* [68] found that baseline BMI and central adiposity (measured by waist circumference, waist-height-ratio, hip circumference, and waist-hip-ratio) predicted the development of diabetes in both genders. Among women, visceral adipose tissue to subcutaneous adipose tissue ratio was found to be a significant predictor for diabetes. In contrast, visceral adipose tissue, waist circumference, BMI, waist-height-ratio, and waist-hip-ratio predicted diabetes development in men. Wang and colleagues [69] reported similar results in that overall obesity (reflected by higher BMI) and central adiposity (reflected by higher waist circumference) predicted the risk for diabetes in men. In brief, these studies showed that central adiposity or visceral fat and BMI are risk factors and predictors for diabetes.

The increased body mass due to obesity is also suggested to be a major contributor to reduced insulin sensitivity in children [70]. Obese adolescent girls were shown to have impaired glucose disposal and failure to increase glucose oxidation and to suppress lipid oxidation in response to insulin infusion [70].

Long term positive energy balance leads to excess triglyceride in the adipose tissue and ectopic triglyceride storage [57]. When adipose tissue is engorged with lipid, there are increased circulating free fatty acids, which eventually cause ectopic lipid accumulation and loss of normal functions and responses in tissues other than adipose tissue. To illustrate, liver steatosis, also known as fatty liver, which is associated with insulin resistance and hyperinsulinemia [71] is commonly seen in obese [57] and type 2 diabetic individuals [72]. Kelley and coworkers [72] reported that fatty liver is correlated to visceral adiposity and more severe insulin resistance in type 2 diabetics as evidenced by significantly increased fasting plasma insulin and fatty acids in comparison to type 2 diabetics without fatty liver. Durante-Mangoni and colleagues [73] found that chronic hepatic C patients with liver steatosis had reduced adiponectin, an adipokine that has been shown to increase insulin sensitivity in the liver and skeletal muscle [74], compared to those without liver steatosis.

Obese individuals have elevated non esterified fatty acids (NEFA), and this impairs normal glucose metabolism. Elevated NEFA causes insulin insensitivity in muscle by reducing insulin-stimulated glucose uptake [75] through the Randle cycle that reduces glucose oxidation [76, 77], and by reducing glucose storage as glycogen [57] through reduced glycogen synthase activity [76]. Elevated NEFA also causes liver insulin insensitivity by increasing hepatic glucose production [75], decreasing insulin-mediated uptake of glucose from the blood, and increasing very-low-density lipoprotein (VLDL) production [57].

Thiazolidinediones (TZDs) and their mechanisms of action

Thiazolidinediones (TZDs) are a class of oral antidiabetic drug that was developed to improve insulin resistance in type 2 diabetes mellitus patients [14]. TZDs improve insulin resistance by increasing hepatic and adipose tissue insulin sensitivity [78]. TZDs are selective agonists of peroxisome proliferator-activated receptor γ (PPARγ) [79], a subset of the subfamily PPARs of the 48-member nuclear-receptor superfamily. PPARγ is a ligand-dependent transcription factor that regulates target gene expression involving glucose and lipid homeostasis [78, 80, 81]. In the cell, PPARγ forms a heterodimer with the retinoid X receptor (RXR) [79, 81, 82]. TZDs have high affinity binding to the PPARγ receptor, which induces conformational change of the heterodimer. This conformation change stabilizes the heterodimer and promotes the binding of PPARγ-RXR complex to the PPARγ response elements (PPRE) in target genes involving cellular differentiation and lipid and glucose metabolism. As a result, it increases target genes transcription and translation [79, 81-83].

PPARγ is found most abundantly in adipose tissue, but also seen in pancreatic β-cells, skeletal muscle, liver, colon, and activated macrophages [78, 79]. PPARγ controls the differentiation and proliferation of adipocytes, fatty acid uptake and storage, and insulin sensitivity in adipocytes [84]. TZDs cause an increase in number of small adipocytes and in subcutaneous adipose tissue mass [78]. When PPARγ is bound to TZDs, it promotes fatty acid uptake and storage in adipose tissue, and this increases adipose tissue mass. This reduces free fatty acid release from adipocytes, and therefore reduces the availability and uptake of free fatty acid by liver cells as well as lowering the triglyceride content in the liver. As a consequence, insulin sensitivity will be increased as elevated plasma free fatty acid concentrations are one of the many contributing factors to insulin resistance.

TZDs also increased the circulating concentrations of adiponectin, an adipokine that is produced by adipose tissue [78]. Serum adiponectin levels are lower in obese and type 2 diabetic individuals, and increased adiponectin level is associated with increased lipid oxidation, improved insulin action, and anti-atherogenic effects [79]. It is also proposed that TZDs increase insulin sensitivity by increasing insulin-stimulated glucose disposal rates in peripheral tissues [84]. TZDs also ameliorate the decline in β-cell function by reducing insulin concentration through their function to increase insulin sensitivity and/or decrease circulating free fatty acids [79].

Due to the many effects of TZDs in various tissues, it is therefore suggested that multiple mechanisms of action are probably involved, such as stimulating adipose tissue to store more free fatty acids, thereby sparing other tissues (liver, skeletal muscle, and possibly β-cells) from lipotoxicity [78], and increasing secretion of adiponectin from adipose tissue as well as glucose disposal rate in peripheral tissue, which increases insulin sensitivity in liver and muscle tissues.

Rosiglitazone effectiveness and side effects

Rosiglitazone is an antidiabetic drug in the class of TZDs. Rosiglitazone has been shown to modulate blood glucose both in animal models of diabetes and in humans [85-88]. Lessard and others [87] found that obese Zucker rats treated with rosiglitazone for a period of 6 weeks had increased body mass and plasma cholesterol, but decreased plasma insulin and triglyceride levels as compared to the control obese rats that were not treated with rosiglitazone. When a glucose tolerance test was performed, obese rats treated with rosiglitazone had improved glucose tolerance shown by reduced glucose area under the curve.

A study called the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) [85] showed that 50.5% of the participants with impaired fasting glucose,

glucose tolerance, or both became normoglycemic after daily administration of 8 mg of rosiglitazone for a median of 3 years. Additionally, Miyazaki and colleagues [86] reported increased hepatic and peripheral tissue insulin sensitivity and reduced NEFA turnover in patients with 12 weeks of rosiglitazone use. They also found that fasting plasma glucose and HbA1c levels of type 2 diabetic patients decreased significantly after the treatment. Also, fasting NEFA and basal NEFA turnover decreased significantly in patients, suggesting there was improved adipose tissue sensitivity to insulin.

In addition, Hanley and colleagues [88] reported a significant improvement in β-cell function in prediabetic subjects that received rosiglitazone treatment over time. Participants with impaired fasting glucose and/or impaired glucose tolerance treated with rosiglitazone for a median of 3 years had improved β-cell function measured by insulinogenic index (IGI) and proinsulin concentration (PI). Moreover, participants had increased IGI / homeostatic model assessment-estimated insulin resistance (HOMA-IR) and reduced PI / C-peptide concentration by 86% and 42%, respectively. These effects persisted even after adjustment for baseline β-cell function.

Despite several studies showing the effectiveness of rosiglitazone in lowering blood glucose, its long-term use has been associated with increased cardiovascular risks. Kang and associates [19] reported that the use of rosiglitazone (4 mg/day) for 120 days resulted in weight gain in type 2 diabetic patients, probably due to adipogenesis. Hussein and colleagues [16] also demonstrated that weight gain is a significant side effect of TZDs treatment. Type 2 diabetic patients given 4 mg or 8 mg rosiglitazone daily for at least 2 months had weight gain of 2.9 kg during the first six months of the study.

Besides weight gain, rosiglitazone is also associated with fluid retention. Hussein and associates [16] reported 21% of the patients treated with rosiglitazone had peripheral edema,

causing 4% of the affected patients to withdraw from receiving treatment. Case studies reviewed by Kermani and Garg [17] also reported pulmonary edema in patients treated with rosiglitazone (4 mg or 8 mg daily), and the discontinuation of rosiglitazone treatment resolved the problem. Another case study by Cekmen and colleagues [18] reported that peripheral edema presented four weeks after the initiation of rosiglitazone, and discontinuation of rosiglitazone treatment improved the condition. The authors suggested that edema may be due to the increased plasma volume and increased renal sodium reabsorption. The increased fluid retention or edema in patients taking rosiglitazone can lead to increased risk for congestive heart failure.

Furthermore, the use of rosiglitazone is associated with other cardiovascular events risk and mortality. Delea and associates [23] reported that the adjusted incidence of heart failure was 8.2% for patients treated with TZDs as compared to 5.3% for control patients. Komajda and associates [24] found that participants treated with metformin or sulfonylurea (oral antidiabetic medications) in combination with rosiglitazone had increased risk of heart failure events compared to participants treated with only metformin or sulfonylurea. Nissen and Wolski [25] reported that the rosiglitazone group had odds ratios of 1.43 for myocardial infarction and 1.64 for death from cardiovascular causes compared to the control group after 24 weeks of treatment.

In addition to weight gain, edema, and heart disease, the use of rosiglitazone was shown to have negative effects on bones. Aubert and colleagues [20] found that there was a 39% higher incidence of fractures in men and women treated with rosiglitazone as compared to the control patients. Patients treated with rosiglitazone had significantly more peripheral fractures as compared to the control group. Berberoglu and colleagues [21] reported a 2.56% bone loss at the trochanter and a 2.18% bone loss at the lumbar spine is associated with each year of rosiglitazone use (4 mg/day) in postmenopausal type 2 diabetic women for a period of two years. Schwartz and colleagues [22] reported that each year use of TZDs was associated with greater bone loss at the

whole body, lumbar spine, and trochanter regions in older type 2 diabetic women during the four year follow-up study.

Combination effects of rosiglitazone with fruits/vegetables on diabetes

As discussed above, rosiglitazone exerts glucose-lowering properties, but also causes detrimental side effects with long-term use. Limited studies have investigated the hypoglycemic effects of combining a pharmacological option such as rosiglitazone with fruits or vegetables with reported glucose-lowering properties. Nivitabishekam and colleagues [89] examined the effects of *Momordica charatia* (bitter melon) in combination with rosiglitazone on lowering blood glucose in rats. Adult male rats were given oral extracts of bitter melon (500 mg/kg body weight dissolved in water), rosiglitazone (2 or 5 mg/kg body weight), and the combination of bitter melon (500 mg/kg body weight) with rosiglitazone (2 or 5mg/kg body weight), and control (1 ml/kg body weight of normal saline) 30 minutes prior to glucose administration. The results of the glucose tolerance test revealed that all treatments showed significant reduction in serum glucose levels when compared to the control group. Both doses of rosiglitazone (2 or 5 mg/kg) combined with bitter melon were more effective in lowering blood glucose compared to their individual treatments. The group administered with the combination of bitter melon with a lower dose of rosiglitazone (2 mg/kg) showed similar glucose-lowering effect as the group given the higher dose of rosiglitazone (5 mg/kg). The greatest effect was seen in the group given the higher dose of rosiglitazone in combination with bitter melon. When similar treatments were given to STZ-induced diabetic rats for a period of 28 days, a similar glucose-lowering effect was seen in all treatment groups. The lower dose of rosiglitazone (2 mg/kg) combined with bitter melon was as effective as the higher dose of rosiglitazone (5 mg/kg) alone in lowering serum glucose levels. Again, the higher dose of rosiglitazone in combination with bitter melon was most efficacious in

lowering blood glucose levels. This study showed that a lower dose of rosiglitazone if combined with natural products with glucose-lowering properties could achieve a similar hypoglycemic effect as the higher dose of rosiglitazone.

In summary, the studies discussed above demonstrate that the use of rosiglitazone, although effective in lowering blood glucose, has numerous side effects. Therefore, alternate glucose-lowering treatments should be explored. The use of a lower dose of rosiglitazone with natural products with hypoglycemic properties like bitter melon is another option to improve glucose control and reduce side effects of the drug.

Fruits and their phytochemicals with glucose-lowering properties

Many studies have focused on the beneficial health effects of fruits and vegetable due to their phytochemicals [90-96]. Phytochemicals have been found to possess a broad range of protective benefits, such as anti-inflammatory effects, prevention of infection, anti-mutagenic effect, and glucose-lowering effects [97].

Studies on the glucose-lowering properties of common fruits (i.e. apples, grapes, blueberries, and plum) and their phytochemicals will be presented in this section. Blueberries and blueberry extract were shown to exert antidiabetic effect on mice [90, 91]. Prior and colleagues [90] reported that mice fed a high fat diet and given purified anthocyanins from blueberry (0.2 mg/mL) in drinking water for a period of 72 days had significantly lower fasting serum glucose concentrations, levels similar to mice fed low fat diet. Another study conducted by DeFuria and colleagues [91] examined the effect of blueberry powder on insulin resistance in mice fed high fat diet also reported similar findings. Mice given a high fat diet (60% of energy from fat) containing 4% blueberry powder were less insulin resistant than mice fed just the high fat diet after 8 weeks of treatment. The area under the curve (AUC) of the glucose tolerance test of the blueberry fed

mice was similar to those fed low fat diet (10% of energy from fat), and had lower blood glucose concentrations compared to the high fat fed mice.

In addition to blueberries, grape seed rich in proanthocyanidin was also found to possess glucose-lowering properties. El-Alfy and colleagues [92] reported that alloxan-induced diabetic rats treated with grape seed proanthocyanidins (100 mg/kg body weight) had significantly lower blood glucose than non-treated diabetic rats at both 48 and 72 hours after the alloxan induction. Also, rats treated with grape seed proanthocyanidins had significantly higher insulin level at 72 hours after the alloxan induction as compared to the non-treated diabetic rats. Therefore, the authors concluded that grape seed proanthocyanidins caused significant reduction in hyperglycemia induced by alloxan. Similarly, Hwang and coworkers [93] examined the effects of grape seed extract on blood glucose in type 2 diabetic mice. Diabetic mice (db/db) gavaged with grape seed extract (50 mg/ kg body weight) for 8 weeks had lower HbA1c level than the non-treated db/db mice, and had significantly lower blood glucose concentrations.

Apples contain high concentrations of flavonoids and a variety of other phytochemicals [98]. Apple consumption may be associated with lower risk of diabetes. Higher quercetin intake, a major component of the apple peel, was also associated with a trend toward decreased risk for type 2 diabetes [94]. Johnston and colleagues [95] examined the effect of apple juice in the acute modification of glucose tolerance. They reported significant reduction in plasma glucose concentration and total AUC of the glucose tolerance test (between 0 and 30 minutes) in participants that consumed apple juice compared to the controls.

Plums contain high concentrations of phenolic compounds, such as flavonoids [96]. Flavonoids have been shown to reduce blood glucose in mice with STZ-induced hyperglycemia, and reduce the risk of type 2 diabetes. Utsunomiya and colleagues [96] found that 1% plum-ekisu (Asian plum juice) mixed with drinking water of Wistar fatty rats significantly decreased food

consumption and body weight at 2 weeks of treatment in comparison to the control rats (water-treated). Plum-treated rats also had significantly lowered plasma glucose levels throughout the glucose tolerance test compared to the control rats, and reduced plasma insulin at 60 minutes after the glucose load. Furthermore, plum (0.25%) reduced plasma cholesterol and increased adiponectin compared to the control rats.

The studies discussed above demonstrate that fruits and their phytochemicals have glucose-lowering effects. Since mango is also a fruit that is rich in phytochemicals, it is therefore a potential natural product to aid in modulating blood glucose and other clinical parameters.

Bioactive compounds in mango

Mango (*Mangifera indica* L.) from the family *Anacardiaceae*, is an evergreen tropical plant [99]. Mango ranks second in terms of internationally traded tropical fruits, and fifth in total world production among major fruit crops [40]. It is cultivated commercially in more than 87 countries, mainly in tropical and subtropical regions [100]. India is the main producing country, followed by China, Thailand, Indonesia, Pakistan, and Mexico [99]. There are hundreds of mango cultivars available worldwide, which all differ in size, color, flavor, seed size, and composition [100]. However, only a few varieties are available in the U.S. market, namely Ataulfo, Francis, Haden, Keitt, Kent, and Tommy Atkins (the common ones) as well as Alphonse, Edward, Kesar, Manila, and Palmer (found only in certain parts of the U.S.) [101].

Mango fruit nutrient and polyphenolic composition varies due to growth condition (climate), variety, harvest conditions, postharvest storage, stages of maturity [102], harvest date, and packaging [103]. To illustrate, total sugars increased and acidity decreased as the fruit ripened [104]. Sucrose content increased 1.3-fold from day 1 to day 19 (day 1 - high firmness, green-colored skin and light yellow pulp; day 19 - very soft texture, slightly reddish-colored skin

and intensely yellow pulp) postharvest, while fructose content increased from day 1 to day 5, followed by a decrease to basal level. Total glucose content, on the other hand, decreased 3-fold from day 1 to day 15. Besides that, vitamin C content decreased as fruit ripened, resulting in a total of 29.75 mg/100 g of reduction from unripe to ripe fruit [105].

In addition to maturity stages, variety also affects mango nutrient and polyphenolic composition. Tommy Atkins variety contained the lowest average β-carotene (4.9 mg/kg mango puree), while the Ataulfo variety contained the highest average β-carotene (26.1 mg/kg mango puree) among the five examined cultivars (Tommy Atkins, Haden, Kent, Keitt, and Ataulfo) [106]. Higher β-carotene content was found in mango harvested at the later harvest dates. The average ascorbic acid concentration for the Tommy Atkins variety was 19.3 mg/100g of puree. Ascorbic acid concentration was either increased or decreased by the harvest dates, but there is no consistent trend to the changes. The average total phenolic content in the Tommy Atkins variety was 27.23 mg of gallic acid equivalent (GAE)/100 g of mango puree, and was similar to Haden, Kent, and Keitt varieties, regardless of harvest date. The Ataulfo variety contained the highest average total phenolic content (109.3 mg of GAE/100 g of mango puree) compared to the other four varieties, regardless of the harvest date. Significantly higher gallotannin contents were found in mango puree harvested at later dates. Ellagic acid and mangiferin contents decreased with later harvest date, except for the Ataulfo variety, which increased with later harvest dates. However, there were large fruit-to-fruit variations within the same cultivar with the same harvest location and date. Singh and colleagues [107] reported higher tannic acid content in ripe compared to raw mango fruit, regardless of variety (Deshi, Langra, Dashahari, Chausa, Amrapali, and Mallika). On the other hand, gallic acid and total phenolics were higher in the raw compared to the ripe fruit, regardless of variety. Overall, phenolic acids including tannic and gallic (at higher amounts), and cinnamic, vanillic, and ferulic acids (at lower amount), differed between varieties.

The condition of mango during harvest can also change nutrient and polyphenolic composition of the mango fruit. Some harvesting methods can cause mechanical injury such as bruising or cuts on the fruit surface [108]. This injury can cause infection and decay in the fruit, which could lead to low pH, sugar, ascorbic acid and low β-carotene concentration, as well as altered amylase and invertase enzyme activity in the mango fruit.

Nutrient and polyphenolic concentration is also influenced by the climate, storage, and packaging. Mangos grown in hotter regions have higher β-carotene content [109]. Storage and packaging also affect nutrient composition in mango. Hymavathi and colleagues [103] found that total carotene and β-carotene content of a mixture of mango powder (Baneshan, Suvarnarekha, Baneshan+Survarnarekha, or Suvarnarekha+Totapuri) decreased significantly during storage regardless of packaging type or variety. The mean total carotene concentration decreased from 5192 µg to 2458 µg (per 100 g powder) in 6 months stored at room temperature (80.6 – 89.6ºF). Nonetheless, overall there was smaller percent loss of total carotene in mango packaged in the metalized polyester (12 µm thickness) with polyethylene (40.2 µm thickness) compared to the polyester poly (40.2 µm thickness). The authors suggested that lower total carotene loss in metalized polyester polyethylene packaging was due to lower permeability of packaging material to oxygen and light. Besides total carotene loss, ascorbic acid also decreased significantly with storage. The authors also suggested this loss could be attributed to the increase in moisture content. The loss of ascorbic acid was more pronounced in mango packaged in the polyester poly (81% loss) than in the metalized polyester polyethylene (53% loss). Metalized polyester polyethylene material absorbed less moisture, thereby protecting ascorbic acid loss in the powder. These findings suggest that regardless of variety, mango powder loses significant amounts of total carotene and ascorbic acid with storage and this can be further exacerbated by packaging.

There are three parts to the mango fruit: the pulp, peel, and seed. Most of the studies on mango have been done using the stem bark and leaves. Because mango pulp is used in this study,

this section will focus on mango pulp. On average, mango pulp contains approximately 1.8 g of fiber, 27.7 mg of vitamin C, 3894 IU of vitamin A, and about 1.12 mg/kg of vitamin E per 100 g of pulp, and significant amounts of polyphenolic compounds, including quercetins, gallotannins, and kaempferols [40]. **Table 1** and **Table 2** show the nutrient composition and polyphenolic compounds in mango pulp, respectively.

Table 1: Nutrient composition of mango pulp [40]

Component	**Amount (per 100g of ripe mango pulp)**
Calories	62.1 – 63.7
Water, g	78.9 – 82.8
Protein, g	0.36 – 0.40
Fat, g	0.30 – 0.53
Carbohydrates, g	16.20 – 17.18
Fiber, g	0.85 – 1.06
Ash, g	0.34 – 0.52
Calcium, mg	6.1 – 12.8
Phosphorus, mg	5.5 – 17.9
Iron, mg	0.20 – 0.63
Vitamin A (carotene), mg	0.135 – 1.872
Thiamine, mg	0.020 – 0.073
Riboflavin, mg	0.025 – 0.068
Niacin, mg	0.025 – 0.707
Ascorbic Acid, mg	7.8 – 172.0
Tryptophan, mg	3 – 6
Lysine, mg	32 – 37
Methionine, mg	4

Table 2: Polyphenol composition in mango pulp

Component	Amount
Total phenols	50 mg GAE/100g fresh weight [110]
Total flavonoids	5.43 mg/g ripe mango pulp flour [111]
Mangiferin	4.6 mg/kg dry matter [112]
Anthocyanins	252.25 mg/g ripe mango pulp flour [111]
Gallic acid	~20 μg/g ripe fresh mango [107]
Tannic acid	~2.4 mg/g ripe fresh mango [107]

Mango and diabetes

Different parts of the mango plant (i.e. stem bark, leaves, peel, seed, and fruit) have been shown to have glucose-lowering properties [27-39]. As discussed earlier, the mango fruit is rich in nutrients and bioactive compounds such as vitamins, minerals, fibers, and phenolic compounds. The next section discusses the studies that examined the glucose-lowering properties of mango.

Mango stem bark and leaf extract

An early study by Aderibigbe and colleagues [27] demonstrated the anti-hyperglycemic effect of mango leaf aqueous extract in rats. At 30 and 60 minutes after glucose loading (1 g/kg body weight of 50% glucose solution), blood glucose levels were significantly reduced in glucose-induced hyperglycemic rats given an aqueous mango leaves extract (1 g/kg body weight) 60 minutes before and simultaneously with glucose loading when compared to the control (without glucose loading) and the oral hypoglycemic agent chlorpropamide (200 mg/kg body weight administered simultaneously or 60 minutes before glucose loading) groups.

Ojewole [28] investigated the hypoglycemic effect of mango stem bark aqueous extract on rats. Both fasted STZ-induced diabetic and normoglycemic rats were administered mango stem bark aqueous extract (50, 100, 200, 400, or 800 mg/kg body weight) orally. Both treated normoglycemic and hyperglycemic rats had significantly lower blood glucose concentrations compared to the distilled water-treated control rats. The author reported that the maximal reduction in the blood glucose levels was at the dose of 800 mg/kg body weight.

A recent study by Bhowmik *et al.* [29] also found beneficial effects of mango leaves and stem bark extract on glucose levels of rats. Mango leaf and stem bark aqueous (0.1 mL/kg body weight) and ethanol extracts (0.125 mL/kg body weight) were given to normoglycemic and STZ-induced diabetic rats 30 minutes prior to and simultaneously with glucose load (2.5 g/kg body weight). Blood was drawn at different time points (0, 60, and 120 minutes for mango extracts given before glucose load; 0, 30, and 75 minutes for mango extracts given simultaneously with glucose load; and 0, 60, and 105 minutes for mango extracts given 30 minutes prior to glucose load). No effect was seen when both extracts were administered before, simultaneously, and prior to glucose load in normoglycemic rats. On the other hand, significant anti-hyperglycemic effect was seen in STZ-induced diabetic rats at 30 and 75 minutes when fed extracts simultaneously with glucose load. Only the stem bark ethanol extract showed significant anti-hyperglycemic effect at 105 minutes when fed before glucose load.

Bhowmik and colleagues [29] examined the effects of mango extracts on intestinal absorption of glucose in fasting normoglycemic and STZ-induced diabetic rats. No significant change was seen in the amount of intestinal absorbed glucose of normoglycemic rats supplemented with either ethanol extracts of stem bark or leaves. However, glucose absorption was reduced (13 - 15% reduction) in STZ-induced diabetic rats supplemented with ethanol extract of stem bark. They concluded that the glucose-lowering effect of mango extracts may be due to

the inhibition of glucose absorption in the gut, and both water and ethanol extracts of stem bark and leaf had significant anti-hyperglycemic effect in STZ-induced diabetic rats.

Morsi and colleagues [30] reported that aqueous extract of mango leaves possesses many beneficial effects. STZ administration resulted in significant body weight loss and increased blood glucose concentration as compared to normal rats. Aqueous leaf extracts (30, 50, and 70 mg/d) given for 42 days significantly reduced body weight loss and blood glucose level in STZ-induced diabetic rats compared to those not given the extract. Other clinical parameters, such as total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides (TG), and plasma high density lipoprotein (HDL) were also improved by the extracts. The authors concluded that mango leaf extracts possessed anti-hyperglycemic activity and produced hypolipidemic effects.

Hossain and others [31] demonstrated antidiabetic and glycogenic effects of the different fractions of ethanol extract of mango leaves (ethyl acetate, chloroform, and petroleum-ether fractions) in normal and alloxan-induced diabetic rats. All fractions of ethanol extract of mango leaves (at a dose of 0.1 ml/100 g of body weight) caused a reduction in fasting blood glucose in alloxan-induced diabetic rats with the petroleum-ether fraction causing the most reduction (88.77%). On the other hand, only the petroleum-ether and the chloroform fractions reduced blood glucose of glucose-induced hyperglycemic rats. Besides lowering blood glucose levels, mango fractions also improved liver glycogen level in alloxan-induced diabetic rats, with the petroleum-ether fraction causing the most improvement (improved to 84% compared to 49% in control diabetic rats). Hence, the authors concluded that fractions of mango leaf extract enhanced liver glycogen synthesis and cellular utilization of glucose, which can both improve glucose homeostasis.

Rawi and colleagues [32] investigated the effects of mango and guava leaf aqueous extracts, and their combination on STZ-induced diabetic rats. Diabetic rats were given mango, guava, mango and guava mixture extract (250 mg/kg body weight), or glibenclamide (0.5 mg/kg body weight) orally, for a period of 4 weeks. The oral glucose tolerance test showed that all treatments significantly decreased blood glucose concentration, when compared to the non-treated diabetic rats. In addition to that, they reported significant reduction in liver glycogen in rats treated with glibenclamide, followed by the mixture, guava, and mango extracts.

In addition to the aqueous extracts from mango leaves and stem bark, mangiferin, a glucosylxanthone phytochemical isolated from mango stem bark and leaf extracts [40], has also been reported to produce hypoglycemic effects. Muruganandan and colleagues [33] investigated the effect of mangiferin, extracted with ethanol from mango leaves, on hyperglycemia and atherogenesis in STZ-induced diabetic rats. They found that rats treated with mangiferin (10 and 20 mg/kg) for a period of 28 days restored the body weight loss due to STZ and the effect was comparable to insulin (6 U/kg, i.p.) treatment. Although not to the same level as insulin, both doses of mangiferin significantly reduced plasma glucose concentrations. Mangiferin also modulated lipid profiles (reduced triglycerides, total cholesterol, and LDL levels, as well as increased HDL levels), comparable to insulin treatment, and significantly reduced the atherogenic index (calculated as (total cholesterol – HDL-C)/HDL-C). An oral glucose tolerance test at 60 minutes after the administration of mangiferin also showed significant improvement. Dineshkumar and colleagues [34] also found similar results. STZ-induced rats treated with ethanol extracted mangiferin (10 or 20 mg/kg) for 30 days significantly restored their lost body weight caused by STZ when compared to the control diabetic rats. Also, the mangiferin group had significant reduction in fasting blood sugar level, TC, TG, and LDL compared to the diabetic control, and an increase in HDL level compared to the glibenclamide group (10 mg/kg). These

findings suggest that mangiferin is an effective hypoglycemic agent that can modulate blood glucose as well as lipid profiles of diabetic animals.

Mango peel and kernel extract

Parmar and Kar [35] reported that mango peel extract had hypoglycemic and hypolipidemic effects in rats. Mango peel methanol extract (200 mg/kg) given orally to rats for 10 days resulted in a significant reduction of serum levels of TC, TG, LDL, and VLDL, and an increase in HDL level as compared to rats fed only atherogenic diet. Furthermore, mango peel extract also caused a significant increase in serum insulin and a decrease in serum glucose when compared to the atherogenic diet fed rats.

Petchi and colleagues [36] reported the hypoglycemic effect of mango kernel extracted with ethanol. Overnight fasted rats fed mango kernel extract orally (200 mg/kg of body weight) showed significant reduction in blood glucose when compared to control rats. Gupta and Gupta [37] also demonstrated similar hypoglycemic effects of mango seed ethanol extract. STZ-induced diabetic rats were given oral mango seed extract (30 mg/kg) for a period of 14 or 21 days. Diabetic rats given mango seed extract for both 14 and 21 days had lower serum glucose compared to the control, and levels similar to diabetic rats treated with glibenclamide, an oral antidiabetic drug. Moreover, mango seed extract treatment (both 14 and 21 days) normalized the HbA1c levels induced by STZ. Serum insulin concentration of rats given mango seed extract (both 14 and 21 days) was also significantly higher than control diabetic rats. In contrast, serum triglyceride and phospholipid were significantly reduced in mango seed extract group in comparison to diabetic control group. These findings showed that both mango peel and kernel extracts were able to modulate blood glucose level in animal model.

Mango pulp

Only a few studies have investigated the glucose-lowering properties of mango pulp. Perpetua and Salgado [38] found that alloxan-induced diabetic rats fed Tommy Atkins variety mango powder for 30 days had significantly lower blood glucose compared to control diabetic rats. Lucas and colleagues [39] also found similar results. Mice receiving the 1% freeze-dried mango (by weight) incorporated into high fat diet for a period of two months had approximately 40 - 90% lower blood glucose in comparison to mice fed high fat diet at 30 - 120 minutes during an oral glucose tolerance test. The total AUC of the glucose tolerance test showed that 1% mango diet was the most effective diet in normalizing blood glucose levels in response to a high fat diet. Furthermore, mice fed the 1% mango diet had similar fasting blood glucose as mice treated with rosiglitazone (50 ppm). In addition to the effect of 1% mango on plasma glucose, they also examined the effects of mango on plasma leptin and adiponectin levels, two cytokines that are produced by adipocytes. Leptin helps to regulate satiety and when in abundance inhibits feeding, and is proinflammatory and platelet proaggregatory [113]. On the other hand, increased concentration of adiponectin is associated with lower incidence of type 2 diabetes, and its level falls with weight gain and in obesity. Adiponectin is anti-inflammatory and potentially anti-atherogenic [113]. Lucas and colleagues [39] reported significantly lower concentration of plasma leptin in mice receiving mango diets (1% and 10% by weight) as compared to mice receiving control and high fat diets. On the other hand, they reported the highest level of plasma adiponectin in mice fed 1% mango diet which was significantly different than mice fed high fat diet. Hence, the authors concluded that a small dose of mango (1% by weight) was more effective than rosiglitazone (approximately 4.33 mg/kg body weight), in lowering glucose level in mice consuming the high fat diet.

The few human studies that examined the effect of mango pulp on postprandial glucose and insulin responses in type 2 diabetic patients also suggested that mango is well tolerated in

diabetics, despite its sweet nature. Roongpisuthipong and colleagues [114] studied the effect of five tropical fruits (pineapple, mango, banana, durian, and rambutan portioned to provide 25 g of digestible carbohydrates) on postprandial glucose and insulin responses of female diabetic patients. Mango and banana had significantly smaller glucose curves than pineapple, rambutan, and durian. However, insulin area of mango was similar to pineapple and was lower than banana. These findings suggest that mango compared to the other four tropical fruits led to a smaller rise in postprandial glucose levels in diabetics.

Similarly, Contractor *et al.* [115] investigated the postprandial glucose response to mango, banana, and sapota (portioned to provide 25 g of digestible carbohydrates) in diabetic patients. They reported that banana caused the highest rise in blood glucose. Mango response peaked at 30 minutes, while banana and sapota peaked at 60 minutes after ingestion. The glucose response with mango, banana, and sapota decreased to basal levels at 3 hours. The glucose AUC of mango and sapota were 76% and 85% of the AUC for banana (100%), respectively; however, they were not significantly different.

Guevarra and Panlasigui [116] studied the effect of chico, mango, pineapple, and papaya fruit (portioned to provide 25 g of digestible carbohydrates) on blood glucose response in type 2 diabetic subjects. They found that chico had the highest carbohydrate content (19.1%) followed by mango (17.3%). The glucose tolerance test showed that chico and mango caused significantly lower blood glucose area than wheat bread (control) in diabetic subjects. Moreover, the glycemic index of chico and mango were lower than pineapple and papaya.

Fatema and colleagues [117] examined serum glucose and insulin response to mango and papaya in type 2 diabetic subjects. Mango and papaya (portioned to provide 50 g digestible carbohydrates) were given to diabetic subjects, and glucose and insulin responses of diabetic subjects were compared to a standard meal (white bread). No significant difference was seen

among bread, papaya, and mango in serum glucose levels of diabetic subjects in fasting and postprandial states. Glycemic index of papaya was similar to that of mango while insulin response to mango was significantly lower than papaya. Thus, the authors suggested that mango instead of papaya should be the recommended food for diabetics.

Yusof *et al.* [118] investigated the effect of ten tropical fruits (mango, rambutan, longan, sapodilla, jackfruits, papaya, watermelon, and three varieties of banana) and four temperate fruits (red apple, orange, grapes, and green pear) on blood glucose response in healthy subjects. All fruits (portioned to provide 50g digestible carbohydrates) were given to healthy subjects, and glucose response was compared to a reference (glucose solution). Grapes, orange, red apple, papaya, and watermelon caused a peak in blood glucose values at 15 minutes, while the other fruits and glucose peaked at 30 minutes. Banana was found to cause the largest rise of blood glucose response (62%) when compared to glucose (100%), whereas green pear caused the lowest rise (18%). The blood glucose response of other fruits was 60% for longan, 59% for rambutan and grapes, 54% for watermelon, 47% for orange, 45% for papaya, 41% for jackfruit, 35% for sapodilla and mango, and 27% for red apple. Hence, the authors concluded that sapodilla and mango (tropical fruits) and green pear and red apple (temperate fruits) are the most suitable fruits to be recommended for diabetic patients.

Both animal and human studies indicate that mango pulp possess some glucose-lowering properties and led to a smaller glucose AUC, which is very similar to the effects of stem bark and leaf aqueous extract. Our earlier study [39] had demonstrated a beneficial effect of mango pulp in modulating clinical parameters in mice fed high fat diet, especially plasma glucose levels. Therefore, we conducted this study to confirm our earlier findings on the positive effect of mango in comparison to the commonly prescribed oral hypoglycemic drug, rosiglitazone. In addition to that, many studies had shown negative effects of rosiglitazone in long-term users. Since mango was previously found to have a hypoglycemic effect, another aim of this study was to determine

whether the addition of mango will lower the dose of rosiglitazone needed to modulate blood glucose and other clinical parameters.

CHAPTER III

METHODOLOGY

Animal treatment and care

Fifty-six two-month old male C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). The animal care and use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Oklahoma State University. Mice were acclimated with standardized rodent pelleted diet (AIN-93M) for a week before dietary treatment began. After acclimation, mice were weighed and randomly divided into seven dietary treatment groups (n=8/group) balanced by initial body weight. Mice were maintained in a 12:12 hour light dark cycle in a temperature controlled room. All high fat-fed groups were fed *ad libitum* throughout the study. The control group (AIN-93M) was fed *ad libitum* for the first four weeks and controlled-feed to the average food intake of high fat-fed groups for the next four weeks. Mice were weighed weekly and had free access to deionized water.

Dietary treatment and composition

Mice were randomly divided into seven dietary treatment groups (n=8/group): a) control (AIN-93M); b) high fat (HF); c) HF + rosiglitazone (50 ppm); d) HF + 1% (w/w) freeze-

dried mango powder; e) HF + 10% (w/w) freeze-dried mango powder; f) HF + 1% freeze-dried mango powder + rosiglitazone (25 ppm); and g) HF + 10% freeze-dried mango powder + rosiglitazone (25 ppm). The AIN-93M diet was purchased from Harlan Teklad (Indianapolis, IN) and contained 75.8%, 9.5% , and 14.7% of calories from carbohydrate, fat, and protein, respectively [119]. The HF diet was based on the formulation of Molnar *et al*. [120] and contained 27.7% carbohydrate, 58.9% fat, and 13.4% protein by calories. Tommy Atkins mangos were purchased from a local grocery store, peeled and the pulp was freeze-dried, ground to powder, analyzed for nutrient composition (**Table 3**), and incorporated at 1% and 10% (w/w) to the HF diets. Tommy Atkins variety was used because it is the most available in the U.S. All HF diets were prepared and mixed in-house and adjusted to have similar macronutrient composition as well as calcium and phosphorus content. The composition of the experimental diets is shown in **Table 4**.

Intraperitoneal glucose tolerance test (IGTT)

Intraperitoneal glucose tolerance tests (IGTTs) were performed after 30 and 60 days of treatment. Mice were fasted overnight but had access to deionized water. After an overnight fast, mice were injected intraperitoneally with 20% D-glucose solution (2 g/kg body weight). Blood samples were obtained from the tail vein at 0, 5, 15, 30, 60, and 120 minutes after glucose injection. Blood glucose concentrations were determined using an AlphaTrak™ glucometer (Abbott Laboratories, North Chicago, IL). The glucose AUC for each dietary treatment group was calculated using the trapezoidal rule [121].

Necropsy and tissue processing

After 60 days on the respective dietary treatments, mice were weighed and fasted for 12 hours before the necropsy. At necropsy, mice were anesthetized with a ketamine/xylazine (25.0:2.5 mg/mL) cocktail (0.15 mL/10 g of body weight). Whole body composition was analyzed using x-ray absorptiometry (GE Lunar PIXImus, Fitchburg, WI). Mice were killed by exsanguination, and blood was collected from the carotid artery into ethylenediaminetetraacetic acid (EDTA) coated tubes Blood was centrifuged for 20 minutes at 4,000 rpm to separate plasma, and plasma was stored at -80°C until further analyses.

White adipose tissue, liver, kidneys, and spleen were weighed, snap-frozen and stored at -80°C until further analyses. A portion of liver and white adipose tissues were fixed in 10% neutral buffered formalin until further analyses.

Clinical analyses

Whole blood glycated hemoglobin (HbA1c), plasma total cholesterol, glucose, high density lipoprotein (HDL), triglycerides (TG), non-esterified fatty acids (NEFA), and fructosamine concentrations were determined using a BioLis24i clinical chemistry analyzer (Carolina Liquid Chemistries Corporation™, Brea, CA). All reagent kits were purchased from Carolina Liquid Chemistries Corporation™ (Brea, CA) except for NEFA (Wako Diagnostics, Richmond, VA).

Serum HbA1c concentrations were determined based on the amount of agglutination formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody added to the plasma. The amount of agglutination is proportional to the amount of HbA1c absorbed on the surface of latex particles, which can be measured as absorbance at 660 nm

wavelength. HbA1c concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma glucose concentrations were measured based on the absorbance at 340 nm wavelength of nicotinamide adenine dinucleotide plus hydrogen (NADH), a by-product of glucose breakdown. The increase in absorbance is directly proportional to glucose concentration in the plasma samples as a glucose molecule will cause a cascade of reactions that produces one micromole of NADH as the by-product. Glucose concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma cholesterol concentrations were measured based on the absorbance of quinoneimine, a dye formed when cholesterol reacted with p-hydroxy benzene sulfonic acid (p-HBS) in the reagent mixture, at 500 nm wavelength. The increase in absorbance due to the formation of quinoneimine is directly proportional to the concentration of cholesterol in the plasma samples. Cholesterol concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma HDL-C concentrations were measured by solubilizing only the HDL lipoprotein particles, so that the HDL cholesterol is released to react with the cholesterol esterase and cholesterol oxidase to give color. The non-HDL lipoproteins are inhibited from reacting with the enzymes. The color that is produced is measured at 600 nm and is proportional to the amount of HDL-C present in the plasma samples. HDL-C concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma triglycerides (TG) were measured based on the absorbance of quinoneimine dye, a product formed when TG reacts with peroxidase. The absorbance of the quinoneimine dye 4-aminophenazone at 505 nm is directly proportional to the concentration of TG in the plasma

samples. TG concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma fructosamine concentrations were measured based on the formation of formazan dye from the reduction of ketoamines to nitroblue tetrazolium (NBT) and to formazan under alkaline condition. The formation of formazan, measured at 505 nm wavelength, is directly proportional to the fructosamine concentration in the plasma samples. Fructosamine concentrations were calculated from a standard curve (Carolina Liquid Chemistries Corporation™, Brea, CA).

Plasma non esterified fatty acids (NEFA) were measured at 550 nm wavelength based on the absorbance of the purple colored end product formed from the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine. The amounts of NEFA in the samples were determined from the optical density and the concentrations were calculated from a standard curve (Wako Diagnostics, Richmond, VA).

Plasma insulin

Plasma insulin concentration was determined using an enzyme-linked immunosorbent assay (ELISA) kit from Millipore ™ Corporation (Billerica, MA). Microtiter plates that were pre-coated with anti-mouse insulin monoclonal antibodies were used. Samples were added to the wells and unbound materials were then washed and the enzyme horseradish peroxidase was added. The unbound or free enzyme conjugates were washed, and the immobilized antibody-enzyme conjugate was determined by reacting with horseradish peroxidase and 3,3',5,5'-tetramethylbenzidine. The reaction was stopped with hydrochloric acid and absorbance was measured spectrophotometrically at 450 nm wavelength and corrected at 590 nm wavelength using a microplate reader (Biotek Synergy HT, Winooski, VT). The absorbance is directly

proportional to the amount of captured molecules (insulin) in the plasma samples. The concentration of insulin was calculated from a standard curve (Millipore ™ Corporation, Billerica, MA).

Liver and fecal total lipid

Snap-frozen liver tissue was ground into powder with a mortar and pestle under liquid nitrogen. Approximately 0.2 gram of the liver powder sample was weighed, transferred into a 50 mL centrifuge tube, and extracted overnight with 25 mL of methanol:chloroform (1:2) solution. Fecal samples were dried, ground, and approximately 1 gram of fecal powder was weighed, and extracted with 10 mL methanol:chloroform (1:2) solution. Determination of liver and fecal total lipid was based on the procedure of Folch *et al.* [122]. Briefly, sulfuric acid (0.05%) was added to the methanol:chloroform solution, vortexed, and allowed to be separated into two phases. After the two phases were separated, the aqueous layer was aspirated off and the volume of the remaining organic layer was recorded. The organic solution was poured into a pre-weighed aluminum pan and had evaporated to dryness under a fume hood. After all the organic solvent had evaporated, pans were again dried in the oven at 100ºC, cooled, and weighed. The amount of liver or fecal total lipid was expressed as lipid or fecal weight (in milligram) per gram of liver or fecal sample.

Liver and adipose tissue histology

Liver and white adipose tissues harvested during necropsy were fixed in 10% neutral buffered formalin solution until further analyses. Liver and white adipose tissues were trimmed and cut into smaller pieces before being processed in a Thermo Scientific Shandon Citadel 1000

tissue processor (Austin, TX). Immediately after processing, tissues were transferred to the paraffin embedding machine (Leica EG1160, Buffalo Grove, IL). Paraffin-embedded tissues were sectioned into 7 μm slices using a high performance microtome (Leica RM2165, Buffalo Grove, IL), mounted onto glass microscope slides, and air-dried. Slides were then washed with alcohol and toluene to remove excess paraffin, stained with hematoxylin and eosin and examined under the Nikon Eclipse TE2000-U Inverted Microscope (Melville, NY) at 10x magnification. The effect of different dietary treatments on white adipose cell sizes and the degree of steatosis in liver tissues were evaluated through observation.

Statistical analyses

All values are presented as mean ± standard deviation. Statistical data were computed and analyzed by one-way ANOVA using SAS software version 9.2 (SAS Institute, Cary, NC). The general linear model (GLM) procedure was used to calculate variance and least square means, while the means were compared using Fisher's least significant difference. Data are considered significantly different with *P* value less than 0.05.

Table 3: Nutrient composition of freeze-dried Tommy Atkins mango used in the study[1]

Parameters	Content/100 g
Calories (kcal)	379
Calories from Total Fat (kcal)	15.8
Fat (g)	1.75
Carbohydrates (g)	85.8
Dietary Fiber (g)	12.9
Protein (g)	5.01
Moisture (g)	4.52
Calcium (mg)	79.2
Phosphorus (mg)	123

[1]Nutrient composition was analyzed by NP Analytical Laboratories (St. Louis, MO).

Table 4: Composition of the experimental diets

Ingredients	AIN-93M[2]	HF[3,4]	HF + 1% Mango[4]	HF + 10% Mango[4]
	(g/kg)			
Mango pulp[1]	0	0	10	100
Total Carbohydrate	721	370	370	370
Cornstarch	465.69	0	0	0
Dextrinized cornstarch	155	100	91.42	14.2
Sucrose	100	270	270	270
Contribution of mango	0	0	8.58	85.8
Total Protein	140	180	180	180
Casein	140	180	179.5	174.99
Contribution of mango	0	0	0.5	5.01
Total Fat	40	350	350	350
Soybean oil	40	40	39.82	38.2
Lard	0	310	310	310
Contribution of mango	0	0	0.18	1.75
Total Fiber	50	50	50	50
Cellulose	50	50	48.71	37.1
Contribution of mango	0	0	1.29	12.9
Vitamin mix (AIN-93-VX)	10	10	10	10
Total mineral mix (AIN-93-MX)	35	35	35	35
Mineral mix (Ca-P deficient)	13.4	13.4	13.4	13.4
Calcium carbonate	12.5	12.5	12.48	12.3
Calcium from mango	0	0	0.0079	0.079
Sodium phosphate, dibasic (NaH_2PO_4)	5.6	4.8	4.73	4.51
Potassium phosphate, monobasic, (KH_2PO4)	2.4	2	2.04	1.94
Phosphorus from mango	0	0	0.012	0.123
Sucrose	1.1	2.3	2.35	2.842
Choline bitartrate	2.5	2.5	2.5	2.5
L-cysteine	1.8	1.8	1.8	1.8
Tert-butylhydroquinone	0.008	0.008	0.008	0.008
Kcal/100g[5]	367	559	547	544

[1]Tommy Atkins variety; purchased from local grocery store, peeled, and pulp was freeze-dried.
[2]AIN-93M contained 75.8%carbohydrate, 9.5%fat, and 14.7%protein by calories [119].
[3]High fat diet formulation containing 27.7%carbohydrate, 58.9%fat, and 13.4%protein by calories [120].
[4]Rosiglitazone was from Cayman Chemical Company (Ann Arbor, MI) and added to the HF diets at a dose of 25 ppm and 50 ppm.
[5]Analyzed by NP Analytical Laboratories (St. Louis, MO).

CHAPTER IV

RESULTS

Food intake, feed efficiency, body and tissue weights

Mice in the AIN group had the highest food intake followed by those in HF+10% mango+25 ppm rosiglitazone, HF+10% mango, and HF+50 ppm rosiglitazone groups (**Table 5**). Mice that received HF, HF+1% mango and HF+1% mango+25 ppm rosiglitazone diets had lower food intakes. A similar pattern was observed with caloric intake (except the AIN group) with mice in the HF+10% mango+25 ppm rosiglitazone, HF+50 ppm rosiglitazone, and HF+10% mango having the highest, followed by HF, HF+1% mango, and HF+1% mango+25 ppm rosiglitazone groups.

Both doses of mango combined with 25 ppm rosiglitazone (HF+1% mango+25 ppm rosiglitazone and HF+10% mango+25 ppm rosiglitazone) had the highest feed efficiency. Among the mice fed HF diets, the rosiglitazone group (HF+50 ppm rosiglitazone) had the lowest feed efficiency, despite having the highest caloric intake. Mice receiving either dose of mango alone (HF+1% mango and HF+10% mango) had statistically similar feed efficiency to that of the HF+50 ppm rosiglitazone group but were also not different from the HF group. As expected, the AIN group had the lowest caloric intake and feed efficiency.

All groups had similar body weight at the initiation of treatment. However, all the HF diets had significantly higher body weights than the AIN diet after only one week of dietary treatment

(**Table 6 & Figure 1**). The HF groups gained approximately three times more weight than the AIN-fed mice (an average of 14 grams vs 5 grams) during the 60 days treatment. Of the HF diets, the HF+10% mango+25 ppm rosiglitazone had the highest weight gain, while the HF+50 ppm rosiglitazone had the lowest weight gain. Mice receiving the mango diets alone (HF+1% mango and HF+10% mango) had statistically similar weight gain and final body weight as the HF+50 ppm rosiglitazone group, but were also still not different from the HF group.

There were no significant differences in the spleen and kidney weights among the treatment groups (**Table 5**). Visceral adipose tissue weight was higher in the HF-fed mice except for the HF+50 ppm rosiglitazone group which was similar to the AIN group. A similar pattern was observed with the liver weight.

Body composition

Percent body fat analyzed by x-ray absorptiometry was consistent with the visceral adipose tissue weight (**Figure 2**). Mice fed AIN and HF+50 ppm rosiglitazone groups had the lowest % body fat and highest % lean body mass as compared to all other dietary treatment groups. The HF+1% mango group had the next lowest % body fat and highest % lean mass, but was also not statistically different from the HF group. The combination of mango with rosiglitazone (HF+1% mango+25 ppm rosiglitazone and HF+10% mango+25 ppm rosiglitazone) had the most negative effect on body composition (higher % body fat and lower % lean mass), bone mineral content and bone mineral density, in which levels were significantly lower than the HF group (**Table 7**).

Intraperitoneal glucose tolerance test (IGTT)

To determine the effect of dietary treatments on glucose clearance, an IGTT was performed after 30 and 60 days of dietary treatment. After 30 days of treatment, there were no significant differences among all the treatment groups in tail blood glucose at baseline and 5 minute after injection of glucose solution (**Figure 3a**). Changes in blood glucose were observed after 15, 30, and 60 minutes of glucose injection with all the HF-fed groups having higher blood glucose than the AIN group. All HF-fed groups had statistically similar blood glucose during these time points. After two hours of glucose injection, blood glucose of the HF-fed groups was similar to the AIN group. The glucose total AUC of all HF-fed groups was similar and significantly higher than AIN group after 30 days of dietary treatment (**Table 8**).

After 60 days, there were significant differences in glucose response, which were not observed after 30 days of treatment (**Figure 3b**). Among the HF-fed mice, baseline blood glucose was not different among all the treatment groups after 30 days, but was different at 60 days. Baseline blood glucose was highest in the HF+1% mango group and lowest in the HF+50 ppm rosiglitazone groups. There were no significant differences in blood glucose among all the groups after 5 minutes of glucose injection. At subsequent time points, only the HF+50 ppm rosiglitazone group had blood glucose concentrations similar to the control group. Additionally, glucose was still different among the treatment groups after two hours post-glucose injection, an observation that is not seen after 30 days of treatment. Total glucose AUC of the AIN and HF+50 ppm rosiglitazone groups was similar and lower than all other treatment groups.

Plasma clinical parameters, liver and fecal total lipids

Statistically, significant differences in plasma lipids (i.e. total and HDL-cholesterol, and NEFA) among treatment groups were observed (**Table 9**). Mice fed the HF+1% mango+25 ppm

rosiglitazone diet had the highest total cholesterol level followed by the HF+10% mango and HF+1% mango groups. These groups did not differ from the HF group. As expected, mice fed the control diet had the lowest total cholesterol. HDL-C had similar pattern to that of total cholesterol with mice fed the HF+10% mango and HF+1% mango+25 ppm rosiglitazone groups having the highest HDL-C concentrations, and the AIN group having the lowest HDL-C. HDL-C levels of mice fed the HF+1% mango and HF+10% mango diets were not different from the HF fed mice. In terms of NEFA level, the HF group had the highest level while the HF+50 ppm rosiglitazone group had the lowest. Mice fed the HF+1% mango and HF+10% mango as well as HF+10% mango+25 ppm rosiglitazone diets had similar NEFA levels to both the HF and AIN groups. There were no significant differences in plasma triglyceride levels among the groups.

Plasma glucose was highest in the HF+1% mango group and statistically similar to the HF group. All the other treatment groups except the HF+1% mango and HF+50 ppm rosiglitazone groups had plasma glucose that was statistically similar to the AIN group. The HF+50 ppm rosiglitazone group had the lowest plasma glucose (**Table 9**). Glycated hemoglobin is significantly higher in mice fed the HF+50 ppm rosiglitazone and was statistically similar to all the mango groups. The AIN group had the lowest HbA1c, which was statistically similar to the HF and HF+1% mango groups. There was no difference in fructosamine concentration among the groups. Mice in the HF+1% mango+25 ppm rosiglitazone and HF+10% mango+25 ppm rosiglitazone groups had the highest insulin level, and was statistically similar to those in the HF and HF+1% mango groups. Insulin level was lowest in the HF+50 ppm rosiglitazone group and statistically similar to the AIN and HF+10% mango groups.

There were no significant differences found in liver total lipid among the treatment groups (**Table 9**). In contrast, fecal total lipid was significantly different with the AIN group having significantly lower fecal total lipid compared to all HF-fed groups. Among the HF-fed groups, the HF+1% mango group had the highest fecal lipid, albeit not statistically different from the HF and HF+50 ppm rosiglitazone groups.

Liver and adipose tissue histology

Figure 4 shows the effects of dietary treatments on the morphology of the white adipose tissue. Adipose tissue histology revealed bigger adipocyte in the HF+1% mango+25 ppm rosiglitazone, and HF+10% mango+25 ppm rosiglitazone groups compared to the other dietary treatment groups. Adipocytes of the AIN and HF+50 ppm rosiglitazone groups were the smallest. Adipocytes of the HF+1% mango and HF+10% mango groups were in between the combination groups and the control group.

Figure 5 shows the effects of dietary treatments on liver histology. Based on visual observation, liver histological image of the HF+1%mango+25ppm rosiglitazone group appeared to have the most lipid droplets (steatosis), followed by the HF group. On the other hand, the least liver steatosis was observed in the HF+50 ppm rosiglitazone group, similar to the AIN group. Some lipid droplets were observed in the livers of the HF+1% mango, HF+10% mango, and HF+10% mango+25 ppm rosiglitazone groups.

Table 5: Effects of dietary treatments on food intake, feed efficiency, initial and final body weights, and tissue weights of mice fed high fat diet for 60 days[1]

	AIN	HF	HF + 1% Mango	HF + 10% Mango	HF + 50 ppm rosiglita-zone	HF + 1% Mango + 25 ppm rosiglita-zone	HF + 10% Mango + 25 ppm rosiglita-zone	*P* Value
Food Intake (g/d)	3.09 ± 0.20^{a}	2.64 ± 0.12^{c}	2.65 ± 0.20^{c}	2.89 ± 0.22^{b}	2.84 ± 0.21^{b}	2.64 ± 0.17^{c}	2.92 ± 0.23^{b}	<0.0001
Calorie Intake (kcal/d)	11.36 ± 0.75^{c}	14.77 ± 0.69^{b}	14.51 ± 1.08^{b}	15.75 ± 1.19^{a}	15.85 ± 1.18^{a}	14.45 ± 0.95^{b}	15.90 ± 1.24^{a}	<0.0001
Feed Efficiency								
(g/g diet)	1.51 ± 0.86^{d}	5.41 ± 1.37^{ab}	4.14 ± 1.62^{bc}	4.60 ± 1.36^{bc}	3.22 ± 0.64^{c}	6.57 ± 1.59^{a}	6.10 ± 1.95^{a}	<0.0001
(g/kcal diet)	0.41 ± 0.23^{d}	1.26 ± 0.32^{ab}	0.97 ± 0.38^{bc}	1.17 ± 0.35^{bc}	0.80 ± 0.16^{c}	1.53 ± 0.37^{a}	1.57 ± 0.50^{a}	<0.0001
Body Weight	**(grams)**							
Initial Weight	21.17 ± 1.92	21.18 ± 1.85	21.25 ± 1.88	21.27 ± 1.55	21.69 ± 1.09	21.35 ± 1.42	21.37 ± 1.37	0.9980
Final Weight	25.85 ± 2.52^{d}	35.47 ± 3.70^{ab}	32.24 ± 4.02^{bc}	34.59 ± 3.97^{bc}	30.83 ± 2.20^{c}	38.71 ± 4.23^{a}	39.20 ± 4.85^{a}	<0.0001
Tissue Weights	**(milligrams)**							
White Adipose	461.4 ± 143.6^{c}	1631.2 ± 521.4^{ab}	1275.7 ± 622.2 ^{b}	1631.2 ± 465.6^{ab}	515.0 ± 251.1^{c}	1768.8 ± 437.4^{a}	1783.8 ± 371.8^{a}	<0.0001
Liver	911.4 ± 169.9^{c}	1095.0 ± 264.5^{abc}	1057.1 ± 85.0^{bc}	1075.7 ± 97.4^{abc}	963.3 ± 137.9^{bc}	1316.2 ± 326.8^{a}	1215.7 ± 391.1^{ab}	0.0433
Spleen	108.6 ± 56.7	87.5 ± 19.8	125.0 ± 75.0	82.5 ± 16.7	105.0 ± 22.6	75.0 ± 26.7	85.0 ± 23.9	0.2052
Kidney	310.0 ± 43.4	350.0 ± 36.6	318.0 ± 28.6	338.8 ± 40.2	320.0 ± 23.7	334.3 ± 29.9	372.5 ± 59.2	0.0681

[1]Values are mean ± SD n = 6-8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other.

Table 6: Effects of dietary treatments on weekly body weight of mice fed high fat diet[1]

	AIN	HF	HF + 1% Mango	HF + 10% Mango	HF + 50 ppm rosiglita-zone	HF + 1% Mango + 25 ppm rosiglita-zone	HF + 10% Mango + 25 ppm rosiglita-Zone	
	(grams)							***P* Value**
Initial weight	21.17 ± 1.92	21.18 ± 1.85	21.25 ± 1.88	21.27 ± 1.55	21.69 ± 1.09	21.35 ± 1.42	21.37 ± 1.37	0.9980
Week 1	22.86 ± 1.50^{c}	24.97 ± 1.42^{ab}	24.76 ± 1.44^{b}	25.20 ± 2.42^{ab}	24.80 ± 1.22^{b}	26.35 ± 1.43^{ab}	26.62 ± 1.80^{a}	0.0015
Week 2	24.14 ± 1.28^{c}	26.89 ± 1.46^{b}	26.37 ± 1.35^{b}	26.33 ± 2.43^{b}	26.11 ± 1.12^{b}	28.80 ± 1.96^{a}	28.99 ± 2.01^{a}	<0.0001
Week 3	23.97 ± 1.46^{d}	28.69 ± 1.95^{bc}	28.16 ± 1.66^{c}	27.96 ± 2.37^{c}	26.88 ± 1.13^{c}	30.82 ± 2.87^{ab}	31.18 ± 2.54^{a}	<0.0001
Week 4	23.78 ± 1.76^{d}	29.90 ± 2.60^{bc}	28.73 ± 2.28^{c}	29.00 ± 2.75^{c}	27.34 ± 1.13^{c}	31.97 ± 3.10^{ab}	32.56 ± 2.82^{a}	<0.0001
Week 5	23.40 ± 1.64^{d}	31.54 ± 3.06^{b}	30.17 ± 2.56^{bc}	30.40 ± 3.28^{bc}	28.28 ± 2.02^{c}	34.78 ± 3.34^{a}	34.71 ± 3.14^{a}	<0.0001
Week 6	23.78 ± 1.79^{d}	32.94 ± 3.41^{b}	31.21 ± 3.10^{bc}	31.40 ± 3.24^{bc}	28.49 ± 1.49^{c}	36.00 ± 3.72^{a}	36.03 ± 3.40^{a}	<0.0001
Week 7	24.19 ± 1.75^{d}	34.40 ± 3.34^{b}	32.10 ± 3.75^{bc}	32.75 ± 3.61^{bc}	29.41 ± 1.60^{c}	37.80 ± 4.21^{a}	38.19 ± 3.78^{a}	<0.0001
Week 8	24.60 ± 1.83^{e}	34.97 ± 3.76^{bc}	32.99 ± 3.86^{cd}	34.24 ± 4.22^{c}	29.78 ± 1.70^{d}	38.27 ± 4.12^{ab}	38.94 ± 4.32^{a}	<0.0001
Final weight	25.85 ± 2.52^{d}	35.47 ± 3.70^{ab}	32.24 ± 4.02^{bc}	34.59 ± 3.97^{bc}	30.83 ± 2.20^{c}	38.71 ± 4.23^{a}	39.20 ± 4.85^{a}	<0.0001
Total weight gain	4.68 ± 2.66^{d}	14.29 ± 3.62^{ab}	10.99 ± 4.31^{bc}	13.32 ± 3.94^{bc}	9.14 ± 1.82^{c}	17.36 ± 4.20^{a}	17.83 ± 5.70^{a}	<0.0001

[1]Values are mean ± SD n = 6-8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other.

Figure 1: Effects of dietary treatments on weekly body weight of mice fed high fat diet

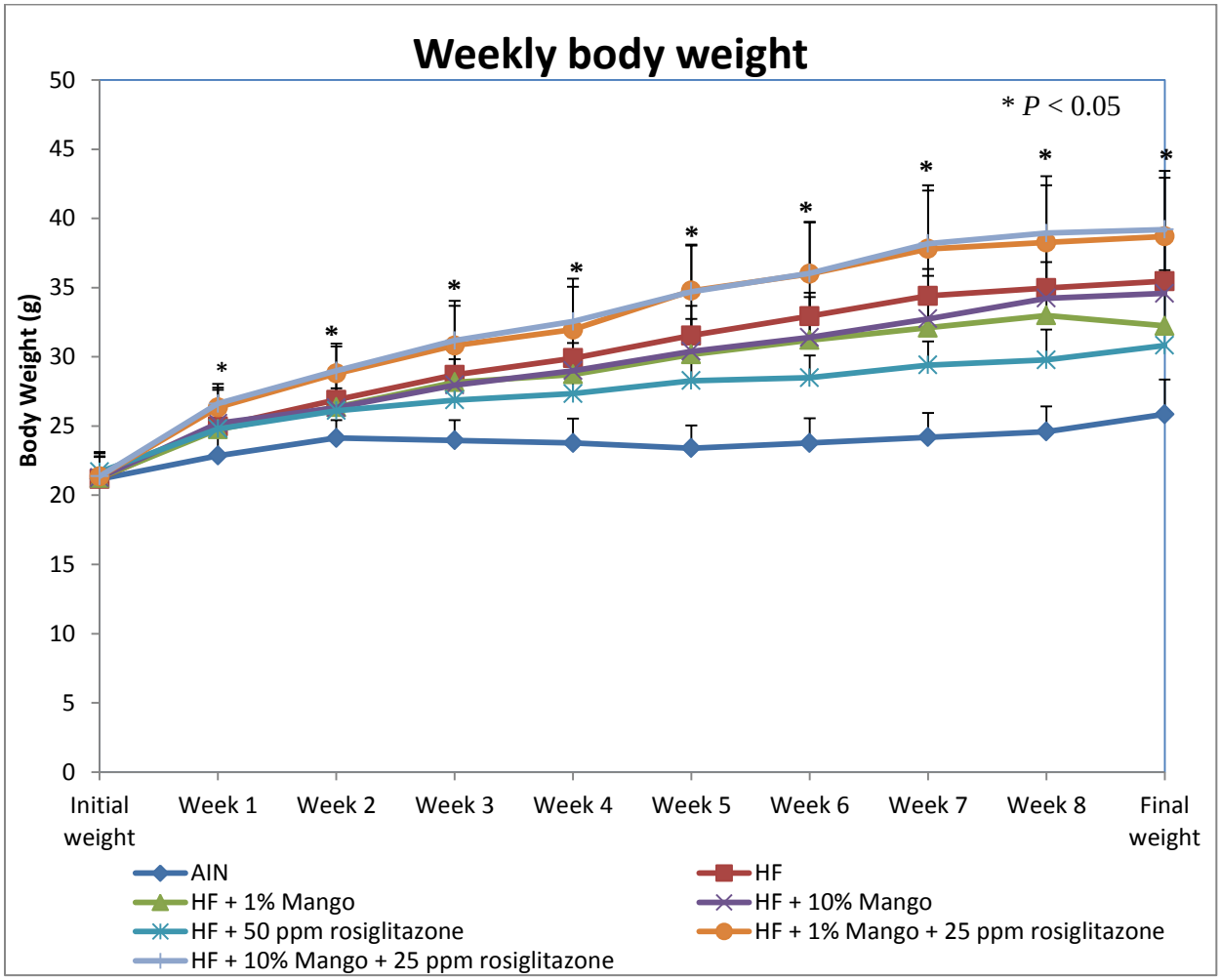


Table 7: Effects of dietary treatments on body composition, bone mineral area (BMA), content (BMC), and density (BMD) of mice fed high fat diet for 60 days[1]

Para-meters	AIN	HF	HF + 1% Mango	HF + 10% Mango	HF + 50 ppm rosiglita-zone	HF + 1% Mango + 25 ppm rosiglita-zone	HF + 10% Mango + 25 ppm rosiglita-zone	*P* value
Lean mass (g)	17.41 ± 1.68^{c}	21.91 ± 1.48^{ab}	20.53 ± 1.85^{b}	21.19 ± 1.93^{ab}	20.95 ± 0.73^{ab}	22.20 ± 1.70^{ab}	22.84 ± 2.48^{a}	<0.0001
Fat mass (g)	5.34 ± 1.12^{d}	11.54 ± 2.44^{bc}	9.46 ± 2.76^{c}	11.11 ± 2.20^{c}	6.60 ± 1.70^{d}	14.35 ± 3.42^{a}	13.98 ± 2.78^{ab}	<0.0001
Total mass (g)	22.72 ± 2.49^{d}	33.48 ± 3.64^{ab}	30.00 ± 3.92^{bc}	32.32 ± 3.79^{b}	27.53 ± 2.25^{c}	36.52 ± 4.53^{a}	36.81 ± 4.73^{a}	<0.0001
BMD (mg/cm²)	50.84 ± 3.27^{a}	50.28 ± 0.95^{a}	49.78 ± 1.93^{ab}	49.59 ± 2.27^{ab}	47.70 ± 1.29^{bc}	47.36 ± 2.02^{c}	48.06 ± 1.90^{bc}	0.0100
BMC (mg)	583.38 ± 57.39^{a}	505.38 ± 26.42^{b}	516.57± 58.89^{b}	505.75 ± 45.75^{b}	493.83 ± 41.66^{b}	422.25 ± 50.90^{c}	430.75 ± 32.33^{c}	<0.0001
BMA (cm²)	11.46 ± 0.50^{a}	10.05 ± 0.49^{b}	10.35 ± 0.89^{b}	10.18 ± 0.57^{b}	10.35 ± 0.78^{b}	8.90 ± 0.75^{c}	8.96 ± 0.41^{c}	<0.0001

[1]Values are mean ± SD n = 6-8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other.

Figure 2: Effects of dietary treatments on % body fat and lean mass of mice fed high fat diet for 60 days

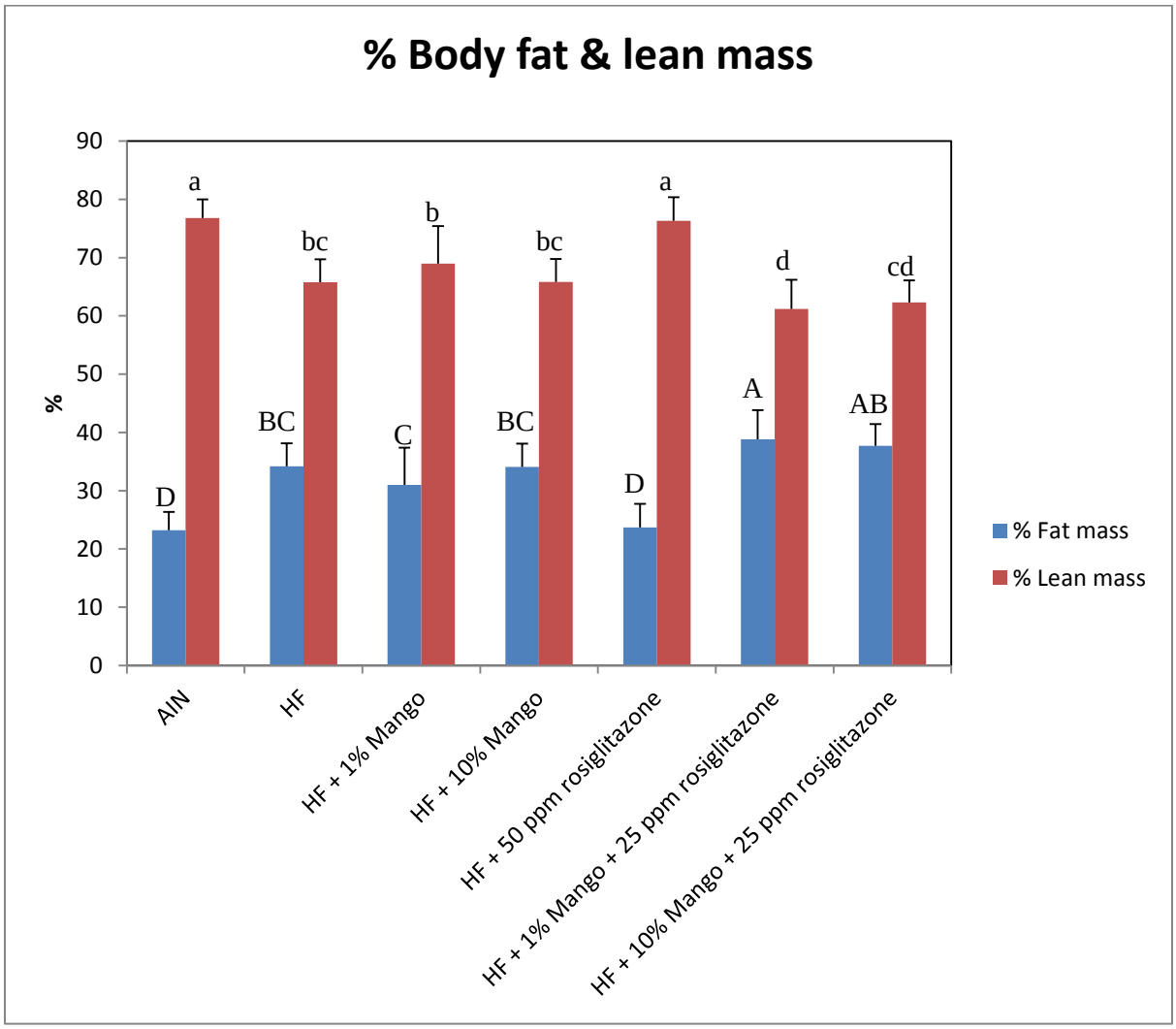
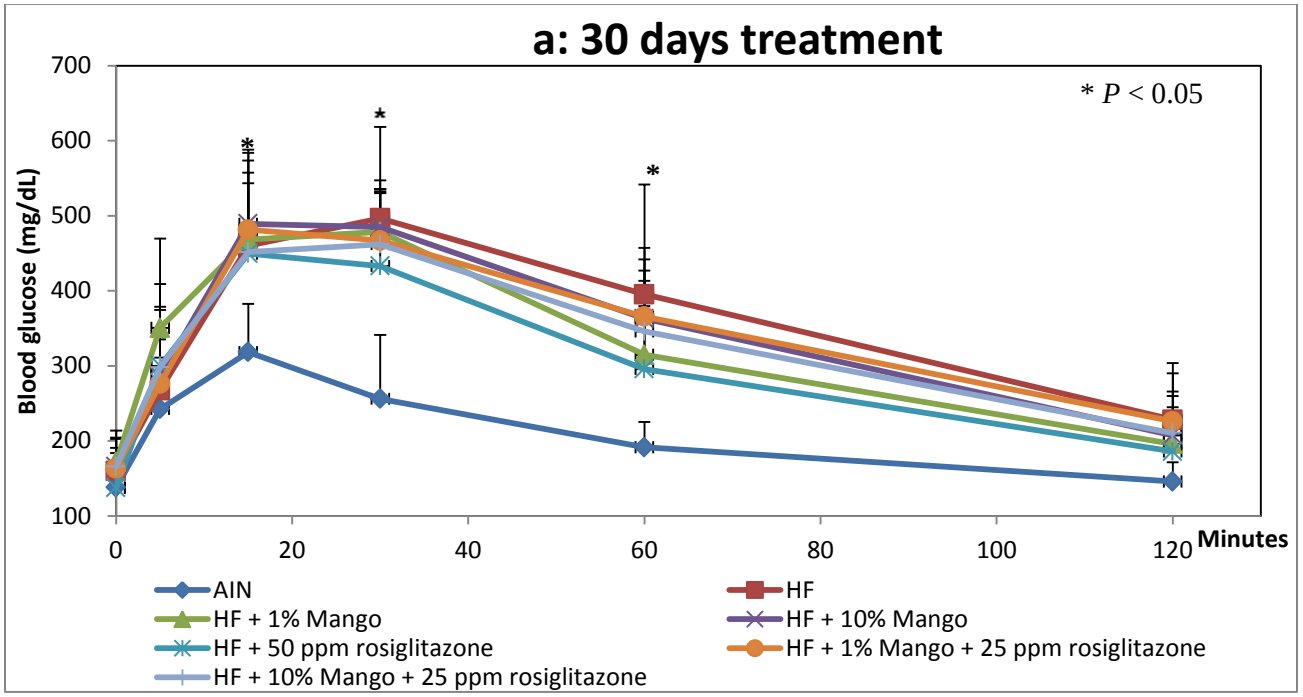


Figure 3 a & b: Effects of dietary treatments on intraperitoneal glucose tolerance test (IGTT) of mice fed high fat diet for 30 days and 60 days



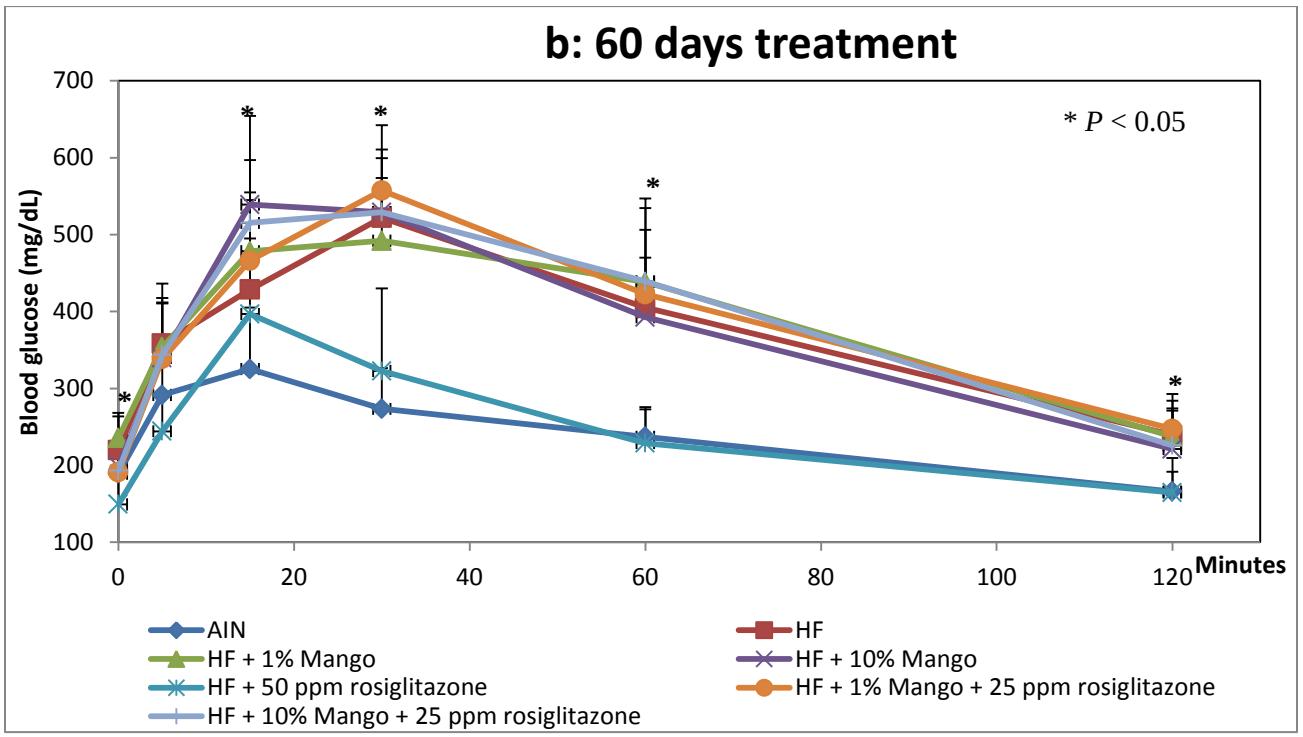


Table 8: Effects of dietary treatments on total glucose area under the curve (AUC) of mice fed high fat diet for 30 days and 60 days[1]

Total AUC	AIN	HF	HF + 1% Mango	HF + 10% Mango	HF + 50 ppm rosiglita-zone	HF + 1% Mango + 25 ppm rosiglita-zone	HF + 10% Mango + 25 ppm rosiglita-zone	
	(mg/dL)minutes							*P* value
30d	24912 ± 4139[b]	43959 ± 8658[a]	39722 ± 8063[a]	42133 ± 5884[a]	36853 ± 8640[a]	42242 ± 8360[a]	40568 ± 6399[a]	<0.0001
60d	28515 ± 3606[b]	45739 ± 5125[a]	47132 ± 5443[a]	45976 ± 9538[a]	29655 ± 7072[b]	47809 ± 7081[a]	47944 ± 7883[a]	<0.0001

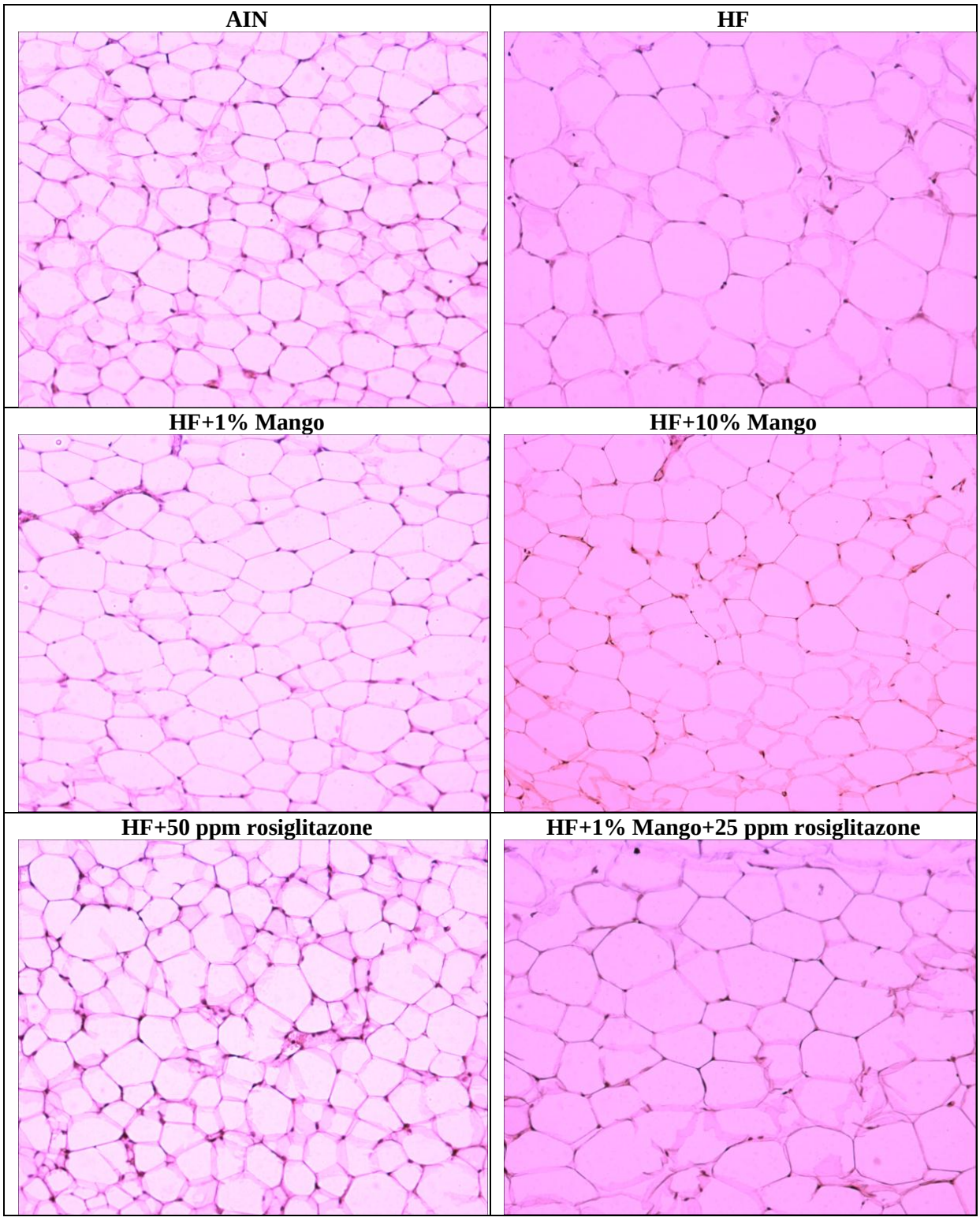
[1]Values are mean ± SD n = 6-8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other.

Table 9: Effects of dietary treatments on plasma clinical parameters, and liver and fecal lipids of mice fed high fat diet for 60 days[1]

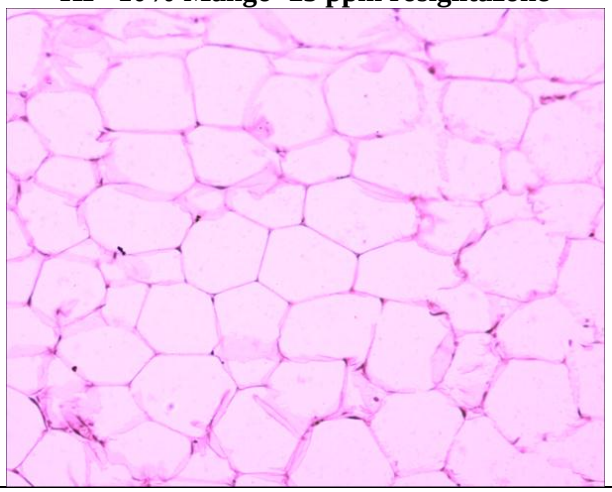
Parameters	AIN	HF	HF + 1% Mango	HF + 10% Mango	HF + 50 ppm rosiglitazone	HF + 1% Mango + 25 ppm rosiglitazone	HF + 10% Mango + 25 ppm rosiglitazone	*P* value
Plasma								
Total cholesterol (mg/dL)	83.62 ± 26.12^{c}	110.19 ± 24.65^{ab}	118.71 ± 36.42^{a}	118.25 ± 18.62^{a}	83.75 ± 5.52^{bc}	125.28 ± 16.54^{a}	107.31 ± 28.62^{abc}	0.0083
Glucose (mg/dL)	189.2 ± 29.0^{b}	219.8 ± 43.7^{ab}	235.6 ± 32.5^{a}	198.8 ± 26.7^{b}	149.2± 48.1^{c}	190.9 ± 27.9^{b}	192.9 ± 27.8^{b}	0.0018
HDL (mg/dL)	31.94 ± 12.88^{c}	43.12 ± 7.02^{ab}	41.64 ± 12.62^{ab}	45.69 ± 3.70^{a}	35.25 ± 2.07^{bc}	45.07 ± 1.77^{a}	38.88 ± 8.53^{abc}	0.0182
Triglycerides (mg/dL)	22.44 ± 2.14	29.88 ± 8.68	25.71 ± 9.94	28.38 ± 4.87	22.83 ± 3.63	27.93 ± 5.27	30.12 ± 6.29	0.1134
Fructosamine (µmol/L)	226.00 ± 68.29	215.25 ± 30.47	182.00 ± 46.21	203.62 ± 25.42	244.50 ± 53.38	217.57 ± 46.76	234.00 ± 33.49	0.2231
NEFA (mEq/L)	0.65 ± 0.12^{abc}	0.74 ± 0.08^{a}	0.64 ± 0.15^{abc}	0.71 ± 0.07^{ab}	0.42 ± 0.06^{d}	0.56 ± 0.09^{c}	0.62 ± 0.08^{bc}	<0.0001
HbA1c (%)	2.96 ± 0.70^{d}	3.42 ± 0.58^{cd}	3.45 ± 0.39^{bcd}	3.94 ± 0.28^{ab}	3.98 ± 0.38^{ab}	3.98 ± 0.43^{a}	3.66 ± 0.34^{abc}	0.0006
Insulin (ng/mL)	0.27 ± 0.03^{bc}	0.34 ± 0.05^{ab}	0.34 ± 0.09^{ab}	0.27 ± 0.01^{bc}	0.24 ± 0.05^{c}	0.36 ± 0.10^{a}	0.40 ± 0.06^{a}	0.0145
Total lipids (mg/g)								
Liver	106.1 ± 77.08	94.6 ± 58.4	171.6 ± 54.3	105.9 ± 53.8	120.0 ± 60.4	171.2 ± 102.8	173.8 ± 96.4	0.2992
Fecal	18.73 ± 3.13^{e}	49.43 ± 2.29^{abc}	54.12 ± 4.57^{a}	37.78 ± 6.57^{d}	52.82 ± 10.62^{ab}	44.53 ± 9.15^{cd}	46.03 ± 3.89^{bcd}	<0.0001

[1]Values are mean ± SD n = 6-8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other.

Figure 4: Effects of dietary treatments on white adipose tissue histology of mice fed high fat diet for 60 days[1]

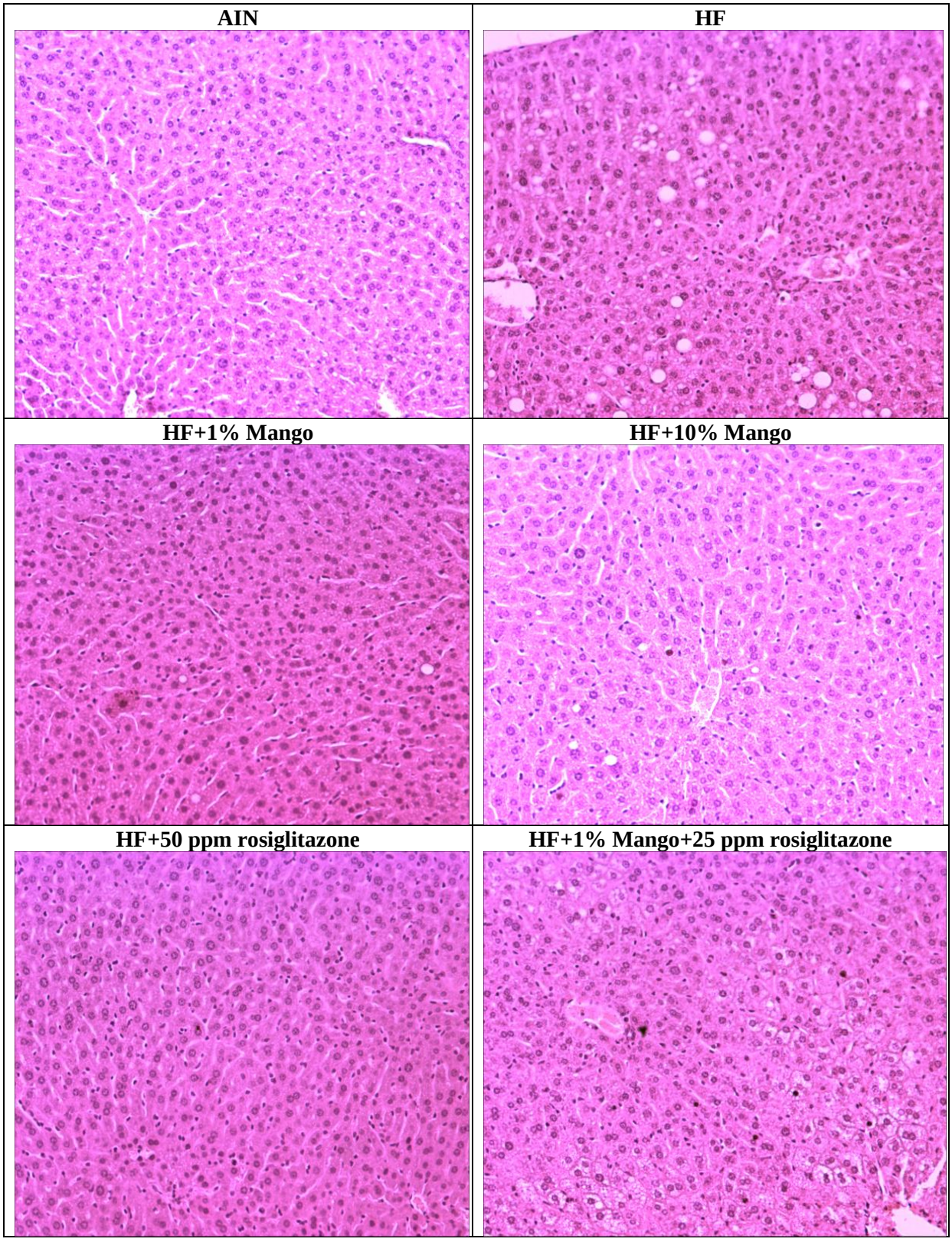


HF+10% Mango+25 ppm rosiglitazone

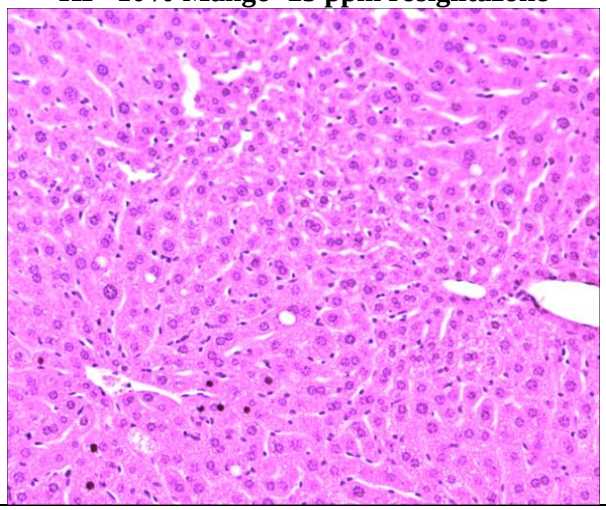


[1]7µm/section; 10 x magnification

Figure 5: Effects of dietary treatments on liver tissue histology of mice fed high fat diet for 60 days[1]



HF+10% Mango+25 ppm rosiglitazone



[1]7µm/section; 10 x magnification

CHAPTER V

DISCUSSION & CONCLUSION

Our earlier study [39] demonstrated that freeze-dried mango supplementation (1% or 10% by weight) for 2 months decreased blood glucose and leptin, increased adiponectin, and improved lipid profiles of mice fed high fat diet. The 1% mango dose had clinical parameters comparable to the control (AIN-93M) and rosiglitazone (50 ppm) diets, and was more effective than the 10% mango dose in modulating the assessed clinical parameters. In the present study, we sought to confirm our earlier findings on the effects of mango in modulating blood glucose level. We also examined whether a lower dose of rosiglitazone (25 ppm) combined with mango would be as efficacious in improving glucose and other clinical parameters as the higher dose of rosiglitazone (50 ppm) alone.

Rosiglitazone, an oral antidiabetic drug, has been shown to be associated with many side effects, including fluid retention [18], cardiovascular events risk [24], weight gain [16], and fractures [20] with long-term use. If mango combined with a lower dose of rosiglitazone is efficacious, this may reduce the side effects associated with long-term use of the higher dose of rosiglitazone. The approach of combining rosiglitazone with fruits/vegetables has been shown to be effective by Nivitabishekam and colleagues [89]. They investigated the glucose-lowering effect of the combination of a lower dose of rosiglitazone (2 mg/kg body weight) with bitter melon. They demonstrated that the lower dose of rosiglitazone in combination with bitter

melon possessed glucose-lowering properties similar to higher dose rosiglitazone (5 mg/kg body weight) alone. This finding indicates the effectiveness of lower dose of rosiglitazone to exert hypoglycemic effects if combined with a functional food such as bitter melon. Based on the findings of Nivitabishekam and colleagues [89] and our earlier findings on the positive effects of mango on blood glucose [39], it is reasonable to assume that a lower dose of rosiglitazone if combined with mango will have a similar hypoglycemic effect as the higher dose of rosiglitazone.

Our findings show that the combinations of the lower dose of rosiglitazone and mango (both at 1% and 10%) were not beneficial in modulating blood glucose. A previous study conducted by Nivitabishekam and colleagues [89] demonstrated that a lower dose of rosiglitazone (2 mg/kg body weight) in combination with bitter melon possessed glucose-lowering properties similar to a higher dose of rosiglitazone (5 mg/kg body weight) alone. The difference in our findings with that of Nivitabishekam and colleagues [89] cannot be explained by the difference in the dose of rosiglitazone. Mice in our combination diet received approximately 1.80 mg of rosiglitazone/kg body weight, which is close to the lower dose (2 mg/kg body weight) used by Nivitabishekam and colleagues [89]. The differences between these two studies may be due to a more potent effect of the bitter melon extract used compared to mango pulp. Nivitabishekam *et al.* [89] used a concentrated extract instead of the whole fruit. The active component of bitter melon extract was likely to be more concentrated than those in the freeze-dried mango pulp used in our study. Additionally, the bitter melon fruit might have an overall better hypoglycemic effect than the mango fruit. In general, there are more studies reporting beneficial effects of bitter melon fruit on lowering blood glucose and lipid profiles [123] compared to only a few studies on mango pulp [38, 39].

In this study, we also did not observe glucose-lowering effect of the mango diets. This finding is not consistent with our earlier results [39] or with Perpetua and Salgado [38] who found significant glucose-lowering effect of various concentrations (1%, 5%, 10%, or 15% by weight)

of Tommy Atkins mango pulp. The difference in findings between the present study and those by Lucas *et al.* [39] and those of Perpetua and Salgado [38] may be due to the stability of the active compounds and nutrient in the mango pulp. In the present study, the mango pulp that was used was a mixture of old freeze-dried mango powder (2007 & 2009) compared to freshly ground freeze-dried mango powder in the study of Lucas *et al.* [39]. A study conducted by Hymavathi *et al.* [103] reported significant losses of total carotene and ascorbic acid with storage at room temperature. A review by Sivakumar *et al.* [108] reported a decrease in gallic acid and gallotannins content during storage. Although the mango powder used in this study was stored in a 4ºC refrigerator, extended storage (>12 months) could still have caused a significant change in the amount of nutrient and bioactive components. Moreover, since we used a mixture of freeze-dried mango powder in the present study, the maturity stage of mangos used may be different. Several investigators have shown that mango nutrient and phenolic composition is affected by the maturity stage [102, 105, 109]. Growth conditions could also be a possible factor that explains these differences between our findings and those by Lucas *et al.* [39] and Perpetua and Salgado [38]. Mercadante and Rodriguez-Amaya [109] found that in general higher β-carotene content was found in mango that was grown in hotter regions. Additionally, Manthey and Perkins-Veazie [106] reported large fruit-to-fruit bioactive component variations within the same cultivar with the same harvest location and date. All the factors mentioned above could help explain the differences in the findings between the present study and those by Lucas *et al.* [39] and Perpetua and Salgado [38].

Our intraperitoneal glucose tolerance test (IGTT) also did not indicate improved glucose clearance with mango or its combination with rosiglitazone in mice fed HF diet. Andrikopoulos and colleagues [124] reported increased fasting plasma glucose and total glucose AUC in high fat-fed mice as compared to chow-fed control mice. Similarly, in the present study, high fat groups (except for the 50 ppm rosiglitazone group) had increased fasting plasma glucose and total

glucose AUC. These results show that high fat diets induced hyperglycemia and that 50 ppm rosiglitazone was able to prevent this high fat diet-induced hyperglycemia. This is not surprising as rosiglitazone has been shown to improve β-cell function [88] and reduce insulin resistance [86]. In contrast, mango (1% and 10%) and its combination with a lower dose of rosiglitazone (25 ppm) were not able to modulate blood glucose in mice fed high fat diets, as shown by significantly higher blood glucose level and total AUC as compared to the control group. Some of the factors discussed above may have contributed to the lack of effect of mango on glucose parameters in this study.

In addition to the mango powder used, there was also difference in the mouse substrain used in our earlier study [39] and this study. Our earlier study [39] used C57BL/6J mice from Harlan Teklad (Indianapolis, IN), whereas C57BL/6 mice from Charles River Laboratories (Wilmington, MA) were used in the present study. C57BL/6J mice have a deletion of exons 7 - 11 in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene that is important for glucose homeostasis and for regulation of insulin secretion, while other substrains of C57BL/6 carry a wildtype *Nnt* allele [125]. Due to the deletion of *Nnt*, C57BL/6J mice exhibit impaired glucose tolerance and reduced insulin secretion, independent of obesity [126]. This gene difference may have contributed to the differences in the findings of the present study and those of Lucas *et al.* [39]. When C57BL/6J male mice were fed high fat (60% fat) diet for a period of 2 and 14 weeks, they had significantly higher peak of plasma blood glucose during a glucose tolerance test in comparison to the C57BL/6NJ [127], a strain similar to C57BL/6 that have an intact *Nnt* allele. Furthermore, C57BL/6J mice at 20 weeks of age when fed a high fat diet had lower weight gain but had significantly higher mean lean and fat mass as compared to C57BL/6NJ mice [127]. Unlike the C57BL/6NJ substrain, C57BL/6J mice when fed a low fat diet (10% fat) did not gain weight after 12 weeks of age [127]. Insulin concentration was also significantly higher in C57BL/6NJ compared to C57BL/6J mice fed low fat diet [127]. Although both strains responded

well to diet-induced obesity, these results show that different substrains of C57BL/6 exhibited differences in glucose homeostasis.

Nevertheless, among the mango-treated groups, the HF+1% mango group trended toward improved body weight and body composition. This finding is very similar to our earlier results [39], in which the 1% mango diet was demonstrated to have the most effect in improving body weight and body composition.

To determine the effects of the different dietary treatments on body weight and body composition, feed efficiency was calculated. The HF groups had higher feed efficiency (an average of 1.22 g/kcal diet) resulting in significantly higher body weight and total body weight gain compared to the control group (0.41 g/kcal diet). This finding is similar to that of Andrikopoulos *et al.* [124], where significantly more weight gain with significantly less food intake was seen in the high fat-fed mice as compared to the chow-fed mice due to higher energy content of the HF diet. Both mango diets caused lower feed efficiency, which was statistically comparable to the HF+50 ppm rosiglitazone group, although still not statistically different from the HF group. Mango may modulate body composition through the PPAR pathway as suggested by Lucas *et al.* [39] as mango contains bioactive components such as mangiferin and quercetin that have been reported to modulate the transactivation of PPAR isoforms [128].

In terms of plasma lipids, high fat diet caused an increase in plasma HDL-C, results that are consistent with that reported by Hayek *et al.*[129]. They explained this phenomenon due to the adaptive mechanism for increased need for HDL cholesterol ester transport rate with high fat feeding. The AIN group had significantly lower HDL-C in comparison with the other dietary treatment groups. Among the high fat-fed groups, the HF+50 ppm rosiglitazone had the lowest HDL-C with a level similar to the AIN group, albeit not significantly different from the HF group. This effect of rosiglitazone on HDL-C is in agreement with the report of Gutschi and colleagues

[130]. They reported decreased HDL-C level in patients given rosiglitazone (4 mg twice daily), and the discontinuation of rosiglitazone increased HDL-C level. Among the mango-treated groups, the HF+10% mango+25 ppm rosiglitazone group had the lowest plasma HDL-C concentration, and was statistically similar to the AIN, HF+50 ppm rosiglitazone, and HF groups. On the other hand, HF+10% mango had the highest plasma HDL-C level, although not statistically different from the HF group. These findings did not show efficacy of mango and its combination with rosiglitazone to modulate HDL-C concentration of mice fed high fat diet.

Schreyer and colleagues [131] reported significant increase in plasma total cholesterol and triglycerides in the high fat or high cholesterol atherogenic diet when compared to the chow diet after 14 days of feeding. Similarly in the present study, plasma total cholesterol was significantly lower in the AIN group as compared to the high fat-fed groups. The HF+50 ppm rosiglitazone group had lower total cholesterol, but was not statistically different from the HF group. There was no significant difference between the mango-treated groups and the HF group in plasma total cholesterol. Although mango was not able to reduce plasma total cholesterol, mango at 1% dose was able to lower plasma free fatty acid (NEFA) to a level that was statistically similar to the AIN group. These findings demonstrate a moderate effect of mango, particularly at 1% dose in modulating lipid parameters. Combining mango with rosiglitazone seems not to provide additional benefit on lipid parameters of mice fed high fat diet.

To determine if the effect of mango on body composition and lipid parameters may be due to decreased fat absorption, fecal lipid was assessed. Fecal lipid was increased in all high fat-fed groups, results that are in agreement with Murase *et al.* [132]. The HF+1% mango group had the highest lipid excretion, followed by the HF+50 ppm rosiglitazone group. Among the high fat-fed groups, final body weight tended to be inversely correlated with fecal lipid level (except for the HF+10% mango group, probably due to higher food intake). This result is similar to Bose and colleagues [133], where they reported higher fecal lipids was inversely correlated with lower

body weight in mice fed high fat diets. In general, groups (HF+1% mango and HF+50 ppm rosiglitazone groups) that had the high fecal lipid also tended to have the most beneficial effect on the assessed clinical parameters. This finding suggests that increased fecal fat excretion could be a factor for some positive effects (i.e. lower white adipose tissue weight and smaller weight gain) observed in the HF+ 1% mango diet on some of the clinical parameters.

In this study, we also examined the changes in the morphology of hepatocytes and adipocytes. Although liver weight as well as liver total lipids of the HF group were not statistically different from the AIN group, histological images of liver tissue of the HF group show some lipid droplets in the hepatocytes, a result similar to reports from Inoue *et al.* [134]. On the contrary, little or no lipid droplet was observed in the hepatocytes of the mice fed the AIN diet. Among the high fat-fed groups, HF+50 ppm rosiglitazone also showed no lipid droplets in the hepatocytes, followed by some in the HF+1% mango, HF+10% mango, and HF+10% mango+25 ppm rosiglitazone groups. This observation may indicate some protective effect on liver steatosis of 50 ppm rosiglitazone and the 1% or 10% mango and 10% mango+25 ppm rosiglitazone diets.

Unlike the liver weight, significant weight gain in the adipose tissue was observed in mice fed high fat diets. Histological images of the HF group also showed bigger adipocyte size when compared to the AIN group. This result is in agreement with Nascimento *et al.* [135], where increased epididymal fat mass and bigger adipocyte sizes were reported in the high fat-high sucrose fed group. Among the high fat-fed groups, the HF+50 ppm rosiglitazone had a significant reduction in adipose tissue weight probably due to its lower feed efficiency and smaller weight gain, followed by the HF+1% mango group albeit not statistically different from the HF group. Histological images of the AIN group revealed uniform and the smallest adipocyte size among all of the dietary treatment groups. As observed in the adipose tissue histology, HF+50 ppm rosiglitazone diet was able to prevent the expansion of adipocytes size induced by high fat

feeding; however, its adipocytes were more varied in shape and size. Similar to the HF+50 ppm rosiglitazone diet, the HF+1% or 10% mango diets were able to prevent the expansion of adipocyte size induced by high fat diet. Besides that, the HF+1% or 10% mango diets led to a more uniform adipocyte size as compared to the HF+50 ppm rosiglitazone diet. In contrast, the HF+1% or 10% mango +25 ppm rosiglitazone diets resulted in the highest adipose tissue weight, albeit not statistically different from the HF group. Correspondingly, histological images of these two groups also revealed bigger adipocytes with varied sizes, demonstrating ineffectiveness of these diets to prevent the increased adipocyte size induced by high fat feeding.

There are several limitations in the present study. As discussed earlier, we combined different batches of mango powder, which had been stored at 4ºC for about 12 months or more; this could have caused significant changes in the amount of the nutrients as well as bioactive components of the mango powder, and might therefore have affected our findings. Our histological data also needs to be interpreted with caution because of its qualitative nature. A blind evaluation or quantification of the histological data may help to avoid bias.

In conclusion, we accept the null hypothesis that a lower dose of rosiglitazone (25 ppm) when combined with mango was not as effective as rosiglitazone alone (50 ppm) in lowering blood glucose level and modulating clinical parameters of mice fed high fat diet. Future work should focus on the use of freshly freeze-dried mango of known origin and maturity. Furthermore, mango and the animal diets should be stored in tightly sealed containers in the freezer during extended storage. There is also a need to determine if mango consumption will improve clinical parameters in humans.

REFERENCES

1. Flegal, K.M., et al., *Prevalence and Trends in Obesity Among US Adults, 1999-2008.* JAMA: The Journal of the American Medical Association, 2010. **303**(3): p. 235-241.
2. World Health Organization. *Obesity and overweight*. 2011; Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.
3. Ogden CL, et al., *Prevalence of obesity in the United States, 2009-2010.* NCHS data brief, no 82. Hyattsville, MD: National Center for Health Statistics., 2012: p. 1-8.
4. Centers for Disease Control and Prevention. *Overweight and obesity*. 2010; Available from: http://www.cdc.gov/obesity/defining.html.
5. Astrup, A., et al., *Obesity as an adaptation to a high-fat diet: evidence from a cross- sectional study.* The American Journal of Clinical Nutrition, 1994. **59**(2): p. 350-355.
6. Must, A., et al., *The Disease Burden Associated With Overweight and Obesity.* JAMA: The Journal of the American Medical Association, 1999. **282**(16): p. 1523-1529.
7. A.D.A.M. Medical Encyclopedia. *Metabolic syndrome: Insulin resistance syndrome; Syndrome X*. 2011; Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0004546/.
8. Sullivan, P.W., et al., *Obesity, Inactivity, and the Prevalence of Diabetes and Diabetes-Related Cardiovascular Comorbidities in the U.S., 2000–2002.* Diabetes Care, 2005. **28**(7): p. 1599-1603.
9. Centers for Disease Control and Prevention. *Diabetes Public Health Resource: Basics about diabetes*. 2012; Available from: http://www.cdc.gov/diabetes/consumer/learn.htm.
10. World Health Organization. *Diabetes*. 2011; Available from: http://www.who.int/mediacentre/factsheets/fs312/en/.
11. Centers for Disease Control and Prevention *National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2011, 2011.
12. American Diabetes Association. *Living With Diabetes*. American Diabetes Association 2012; Available from: http://www.diabetes.org/living-with-diabetes/treatment-and-care/medication/oral-medications/what-are-my-options.html.
13. Lorenzati, B., et al., *Oral Hypoglycemic Drugs: Pathophysiological Basis of Their Mechanism of ActionOral Hypoglycemic Drugs: Pathophysiological Basis of Their Mechanism of Action.* Pharmaceuticals, 2010. **3**(9): p. 3005-3020.

14. A.D.A.M. Medical Encyclopedia. *Thiazolidinediones*. 2011; Available from: http://www.nlm.nih.gov/medlineplus/ency/imagepages/19829.htm.
15. Nesto, R.W., et al., *Thiazolidinedione Use, Fluid Retention, and Congestive Heart Failure.* Diabetes Care, 2004. **27**(1): p. 256-263.
16. Hussein, Z., et al., *Effectiveness and side effects of thiazolidinediones for type 2 diabetes: real-life experience from a tertiary hospital.* Medical Journal of Australia, 2004. **181**(10): p. 536-539.
17. Kermani, A. and Garg, A., *Thiazolidinedione-Associated Congestive Heart Failure and Pulmonary Edema.* Mayo Clinic Proceedings, 2003. **78**(9): p. 1088.
18. Çekmen, N., et al., *Acute Pulmonary Edema Due to Rosiglitazone Use in a Patient With Diabetes Mellitus.* Journal of Intensive Care Medicine, 2006. **21**(1): p. 47-50.
19. Kang, E.S., et al., *The 11482G>A Polymorphism in the Perilipin Gene Is Associated With Weight Gain With Rosiglitazone Treatment in Type 2 Diabetes.* Diabetes Care, 2006. **29**(6): p. 1320-1324.
20. Aubert, R.E., et al., *Rosiglitazone and pioglitazone increase fracture risk in women and men with type 2 diabetes.* Diabetes, Obesity and Metabolism, 2010. **12**(8): p. 716-721.
21. Berberoglu, Z., Yazici, A.C., and Demirag, N.G., *ORIGINAL ARTICLE: Effects of rosiglitazone on bone mineral density and remodelling parameters in Postmenopausal diabetic women: a 2-year follow-up study.* Clinical Endocrinology, 2010. **73**(3): p. 305-312.
22. Schwartz, A.V., et al., *Thiazolidinedione Use and Bone Loss in Older Diabetic Adults.* Journal of Clinical Endocrinology & Metabolism, 2006. **91**(9): p. 3349-3354.
23. Delea, T.E., et al., *Use of Thiazolidinediones and Risk of Heart Failure in People With Type 2 Diabetes.* Diabetes Care, 2003. **26**(11): p. 2983-2989.
24. Komajda, M., et al., *Heart failure events with rosiglitazone in type 2 diabetes: data from the RECORD clinical trial.* European Heart Journal, 2010. **31**(7): p. 824-831.
25. Nissen, S.E. and Wolski, K., *Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes.* New England Journal of Medicine, 2007. **356**(24): p. 2457-2471.
26. Singh, S., Loke, Y.K., and. Furberg, C.D, *Long-term Risk of Cardiovascular Events With Rosiglitazone.* JAMA: The Journal of the American Medical Association, 2007. **298**(10): p. 1189-1195.
27. Aderibigbe, A.O., Emudianughe, T.S, and Lawal, B.A.S., *Antihyperglycaemic effect of Mangifera indica in rat.* Phytotherapy Research, 1999. **13**(6): p. 504-507.
28. Ojewole, J.A.O., *Antiinflammatory, analgesic and hypoglycemic effects of Mangifera indica Linn. (Anacardiaceae) stem-bark aqueous extract.* Methods Find Exp Clin Pharmacol, 2005. **27**(8): p. 547-554.
29. Bhowmik, A., et al., *Studies on the antidiabetic effects of Mangifera indica stem-barks and leaves on nondiabetic, type 1 and type 2 diabetic model rats*. 2009. Vol. 4. 2009.

30. Morsi, R., El-Tahan, N., and El-Hadad, A., *Effects of aqueous extract mangifera indica leaves, as functional foods.* Journal of Applied Sciences Research, 2010. **6**(6): p. 712-721.
31. Hossain, M.S., et al., *Antidiabetic and Glycogenesis Effects of Different Fractions of Ethanolic Extract of Leaves of Mangifera indica (Linn.) in Normal and Alloxan Induced Diabetic Rats.* Journal of Medical Sciences, 2010. **10**(4): p. 80-86.
32. Rawi, S.M., Mourad, I.M, and Sayed, D.A., *Biochemical changes in experimental diabetes before and after treatment with mangifera indica and psidium guava extract.* International Journal of Pharma and Bio Sciences, 2011. **2**(2): p. 29-41.
33. Muruganandan, S., et al., *Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats.* Journal of Ethnopharmacology, 2005. **97**(3): p. 497-501.
34. Dineshkumar, B., Mitra, A., and Manjunatha, M., *Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (Xanthone Glucoside) in streptozotocin-induced Type 1 and Type 2 diabetic model rats*. 2011. Vol. 1. 2011.
35. Parmar, H.S. and Kar, A., *Possible amelioration of atherogenic diet induced dyslipidemia, hypothyroidism and hyperglycemia by the peel extracts of Mangifera indica, Cucumis melo and Citrullus vulgaris fruits in rats.* Biofactors, 2008. **33**(1): p. 13-24.
36. Petchi, R., et al., *Antidiabetic effect of kernel seeds extract of Mangifera indica (Anacardiaceae).* Inernational Journal of Pharma and Bio Sciences, 2011. **2**(1): p. 385-393.
37. Gupta, R. and Gupta, R.S., *Antidiabetic efficacy of Mangifera Indica seed kernels in rats: a comparative study with glibenclamide.* Diabetologica Croatica, 2011. **40**(4): p. 107-112.
38. Perpétuo, G.F. and Salgado, J.M., *Effect of mango (Mangifera indica, L.) ingestion on blood glucose levels of normal and diabetic rats.* Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 2003. **58**(3): p. 1-12.
39. Lucas, E.A., et al., *Mango modulates body fat and plasma glucose and lipids in mice fed a high-fat diet.* British Journal of Nutrition, 2011. **106**(10): p. 1495-1505.
40. Masibo, M. and He, Q., *Mango Bioactive Compounds and Related Nutraceutical Properties—A Review.* Food Reviews International, 2009. **25**(4): p. 346-370.
41. Masibo, M. and. He, Q., *Major Mango Polyphenols and Their Potential Significance to Human Health.* Comprehensive Reviews in Food Science and Food Safety, 2008. **7**(4): p. 309-319.
42. Huang, E.S., et al., *Projecting the Future Diabetes Population Size and Related Costs for the U.S.* Diabetes Care, 2009. **32**(12): p. 2225-2229.
43. Bate, K.L. and Jerums, G., *Preventing complications of diabetes.* The Medical Journal of Australia, 2003. **179**(498-503).
44. Young, B.A., et al., *Diabetes Complications Severity Index and Risk of Mortality, Hospitalization, and Healthcare Utilization.* American Journal of Managed Care, 2008. **14**(1): p. 15-24.
45. King, K.D., Jones, J.D., and Warthen, J., *Microvascular and macrovascular complications of diatebes mellitus.* American Journal of Pharmaceutical Education, 2005. **69**(5): p. 1-5.

46. A.D.A.M. Medical Encyclopedia. *Diabetes and eye disease: Retinopathy - diabetic; Photocoagulation - retina; Diabetic retinopathy*. 2012; Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0002192/.
47. The Eye Diseases Prevalence Research Group, *The Prevalence of Diabetic Retinopathy Among Adults in the United States.* Arch Ophthalmol, 2004. **122**(4): p. 552-563.
48. Fowler, M.J., *Microvascular and Macrovascular Complications of Diabetes.* Clinical Diabetes, 2011. **29**(3): p. 116-122.
49. Saaddine, J.B., et al., *Projection of Diabetic Retinopathy and Other Major Eye Diseases Among People With Diabetes Mellitus: United States, 2005-2050.* Arch Ophthalmol, 2008. **126**(12): p. 1740-1747.
50. Adiga, S., Adiga, U., and Lin, J.T.G., *Complications of diabetes mellitus and glucemic control.* Journal of Global Pharma Technology, 2010. **2**(7): p. 60-63.
51. A.D.A.M. Medical Encyclopedia. *Diabetes and kidney disease: Kimmelstiel-Wilson disease; Diabeteic glomerulosclerosis; Nephropathy - diabetic; Diabetic nephropathy*. 2012; Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001524/.
52. *Nephropathy in Diabetes.* Diabetes Care, 2004. **27**(suppl 1): p. s79-s83.
53. Tesfaye, S., *Neuropathy in diabetes.* 2010. **38**(12): p. 649-655.
54. A.D.A.M. Medical Encyclopedia. *Diabetic neuropathy: Nerve damage - diabetic*. 2011; Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001713/.
55. A.D.A.M. Medical Encyclopedia. *Type 2 diabetes: Noninsulin-dependent diabetes; Diabetes - type 2; Adult-onset diabetes*. 2011; Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001356/.
56. Codario, R.A., *Pathophysiology of Type 2 Diabetes, Pre-Diabetes, and the Metabolic Syndrome*, 2011, Humana Press. p. 1-14.
57. Scheen, A.J., *Pathophysiology of type 2 diabetes.* Acta Clinica Belgica, 2003. **58**(6): p. 335-341.
58. Shepherd, P.R. and Kahn, B.B., *Glucose transporters and insulin action.* The New England Journal of Medicine, 1999. **341**(4): p. 248-57.
59. Surampudi, P.N., John-Kalarickal, J., and Fonseca, V.A., *Emerging Concepts in the Pathophysiology of Type 2 Diabetes Mellitus.* Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine, 2009. **76**(3): p. 216-226.
60. Arner, P., *Insulin resistance in type 2 diabetes: role of fatty acids.* Diabetes/Metabolism Research and Reviews, 2002. **18**(S2): p. S5-S9.
61. Lam, T.K.T., et al., *Mechanisms of the free fatty acid-induced increase in hepatic glucose production.* American Journal of Physiology - Endocrinology And Metabolism, 2003. **284**(5): p. E863-E873.
62. Hue, L. and Taegtmeyer, H., *The randle cycle revisited: a new head for an old hat.* Am J Physiol Endocrinol Metab, 2009. **297**: p. E578-E591.
63. Thanopoulou, A.C., et al., *Dietary Fat Intake as Risk Factor for the Development of Diabetes.* Diabetes Care, 2003. **26**(2): p. 302-307.
64. Marshall, J.A., et al., *Dietary Fat Predicts Conversion From Impaired Glucose Tolerance to NIDDM: The San Luis Valley Diabetes Study.* Diabetes Care, 1994. **17**(1): p. 50-56.

65. Salmerón, J., et al., *Dietary fat intake and risk of type 2 diabetes in women.* The American Journal of Clinical Nutrition, 2001. **73**(6): p. 1019-1026.
66. Centers for Disease Control and Prevention, *Prevalence of Overweight and Obesity Among Adults with Diagnosed Diabetes --- United States, 1988--1994 and 1999--2002*, 2004: Morbidity and Mortality Weekly Report. p. 1066-1068.
67. Mokdad, A.H., et al., *Prevalence of Obesity, Diabetes, and Obesity-Related Health Risk Factors, 2001.* JAMA: The Journal of the American Medical Association, 2003. **289**(1): p. 76-79.
68. Bray, G.A., et al., *Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program.* The American Journal of Clinical Nutrition, 2008. **87**(5): p. 1212-1218.
69. Wang, Y., et al., *Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men.* The American Journal of Clinical Nutrition, 2005. **81**(3): p. 555-563.
70. Goran, M.I., Ball, G.D.C., and Cruz, M.L., *Obesity and Risk of Type 2 Diabetes and Cardiovascular Disease in Children and Adolescents.* Journal of Clinical Endocrinology & Metabolism, 2003. **88**(4): p. 1417-1427.
71. Marchesini, G., et al., *Association of nonalcoholic fatty liver disease with insulin resistance.* The American Journal of Medicine, 1999. **107**(5): p. 450-455.
72. Kelley, D.E., et al., *Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance.* American Journal of Physiology - Endocrinology And Metabolism, 2003. **285**(4): p. E906-E916.
73. Durante-Mangoni, E., et al., *Hepatic steatosis and insulin resistance are associated with serum imbalance of adiponectin/tumour necrosis factor-α in chronic hepatitis C patients.* Alimentary Pharmacology & Therapeutics, 2006. **24**(9): p. 1349-1357.
74. Kadowaki, T., et al., *Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome.* The Journal of Clinical Investigation, 2006. **116**(7): p. 1784-1792.
75. Leong, K.S. and Wilding, J.P., *Obesity and diabetes.* Best Practice & Research Clinical Endocrinology & Metabolism, 1999. **13**(2): p. 221-237.
76. Boden, G. and Shulman, G.I., *Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction.* European Journal of Clinical Investigation, 2002. **32**: p. 14-23.
77. Bergman, R.N. and Ader, M., *Free Fatty Acids and Pathogenesis of Type 2 Diabetes Mellitus.* Trends in Endocrinology & Metabolism, 2000. **11**(9): p. 351-356.
78. Yki-Järvinen, H., *Thiazolidinediones.* New England Journal of Medicine, 2004. **351**(11): p. 1106-1118.
79. Greenfield, J.R. and Chisholm, D.J., *Thiazolidinediones-mechanisms of action.* Australian Prescriber, 2004. **27**(3): p. 67-70.
80. Wilding, J., *Thiazolidinediones, insulin resistance and obesity: finding a balance.* International Journal of Clinical Practice, 2006. **60**(10): p. 1272-1280.
81. Berger, J. and Moller, D.E., *The mechanisms of action of PPARs.* Annual Review of Medicine, 2002. **53**: p. 409-435.

82. Hauner, H., *The mode of action of thiazolidinediones.* Diabetes/Metabolism Research and Reviews, 2002. **18**(S2): p. S10-S15.
83. Reginato, M.J. and Lazar, M.A., *Mechanisms by which Thiazolidinediones Enhance Insulin Action.* Trends in Endocrinology & Metabolism, 1999. **10**(1): p. 9-13.
84. Schoonjans, K. and Auwerx, J., *Thiazolidinediones: an update.* The Lancet, 2000. **355**(9208): p. 1008-1010.
85. *Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial.* The Lancet, 2006. **368**(9541): p. 1096-1105.
86. Miyazaki, Y., et al., *Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in Type II diabetic patients.* Diabetologia, 2001. **44**(12): p. 2210-2219.
87. Lessard, S.J., et al., *Rosiglitazone Enhances Glucose Tolerance by Mechanisms Other than Reduction of Fatty Acid Accumulation within Skeletal Muscle.* Endocrinology, 2004. **145**(12): p. 5665-5670.
88. Hanley, A.J., et al., *Effect of Rosiglitazone and Ramipril on β-Cell Function in People With Impaired Glucose Tolerance or Impaired Fasting Glucose.* Diabetes Care, 2010. **33**(3): p. 608-613.
89. Nivitabishekam, S.N., Asad, M., and Prasad, V.S., *Pharmacodynamic interaction of Momordica charantia with rosiglitazone in rats.* Chemico-Biological Interactions, 2009. **177**(3): p. 247-253.
90. Prior, R.L., et al., *Purified Blueberry Anthocyanins and Blueberry Juice Alter Development of Obesity in Mice Fed an Obesogenic High-Fat Diet†.* Journal of Agricultural and Food Chemistry, 2010. **58**(7): p. 3970-3976.
91. DeFuria, J., et al., *Dietary Blueberry Attenuates Whole-Body Insulin Resistance in High Fat-Fed Mice by Reducing Adipocyte Death and Its Inflammatory Sequelae.* The Journal of Nutrition, 2009. **139**(8): p. 1510-1516.
92. El-Alfy, A.T., Ahmed, A.A.E., and Fatani, A.J., *Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats.* Pharmacological Research, 2005. **52**(3): p. 264-270.
93. Hwang, I.K., et al., *Effects of grape seed extract and its ethylacetate/ethanol fraction on blood glucose levels in a model of type 2 diabetes.* Phytotherapy Research, 2009. **23**(8): p. 1182-1185.
94. Knekt, P., et al., *Flavonoid intake and risk of chronic diseases.* The American Journal of Clinical Nutrition, 2002. **76**(3): p. 560-568.
95. Johnston, K.L., Clifford, M.N., and, Morgan, L.M., *Possible role for apple juice phenolic compounds in the acute modification of glucose tolerance and gastrointestinal hormone secretion in humans.* Journal of the Science of Food and Agriculture, 2002. **82**(15): p. 1800-1805.
96. Utsunomiya, H., et al., *Anti-hyperglycemic effects of plum in a rat model of obesity and type 2 diabetes, Wistar fatty rat.* Biomedical Research, 2005. **26**(5): p. 193-200.
97. Dillard, C.J. and German, J.B., *Phytochemicals: nutraceuticals and human health.* Journal of the Science of Food and Agriculture, 2000. **80**(12): p. 1744-1756.

98. Boyer, J. and Liu, R., *Apple phytochemicals and their health benefits.* Nutrition Journal, 2004. **3**(1): p. 5.
99. Tharanathan, R.N., Yashoda, H.M., and Prabha, T.N., *Mango (Mangifera indica L.), "The King of Fruits"—An Overview.* Food Reviews International, 2006. **22**(2): p. 95-123.
100. Barreto, J.C., et al., *Characterization and Quantitation of Polyphenolic Compounds in Bark, Kernel, Leaves, and Peel of Mango (Mangifera indica L.).* Journal of Agricultural and Food Chemistry, 2008. **56**(14): p. 5599-5610.
101. National Mango Board. *Varieties and availability*. 2011; Available from: http://www.mango.org/varieties-and-availability.
102. Gil, A.M., et al., *Study of the Compositional Changes of Mango during Ripening by Use of Nuclear Magnetic Resonance Spectroscopy.* Journal of Agricultural and Food Chemistry, 2000. **48**(5): p. 1524-1536.
103. Hymavathi, T.V. and Khader, V., *Carotene, ascorbic acid and sugar content of vacuum dehydrated ripe mango powders stored in flexible packaging material.* Journal of Food Composition and Analysis, 2005. **18**(2–3): p. 181-192.
104. Medlicott, A.P. and Thompson, A.K., *Analysis of sugars and organic acids in ripening mango fruits (Mangifera indica L. var Keitt) by high performance liquid chromatography.* Journal of the Science of Food and Agriculture, 1985. **36**(7): p. 561-566.
105. Gowda, I.N. and Huddar, A.G., *Studies on ripening changes in mago (mangifera indica L.) fruits.* J. Food Sci. Technol, 2001. **38**(2): p. 135-137.
106. Manthey, J.A. and Perkins-Veazie, P., *Influences of Harvest Date and Location on the Levels of β-Carotene, Ascorbic Acid, Total Phenols, the in Vitro Antioxidant Capacity, and Phenolic Profiles of Five Commercial Varieties of Mango (Mangifera indica L.).* Journal of Agricultural and Food Chemistry, 2009. **57**(22): p. 10825-10830.
107. Singh, U.P., et al., *Characterization of phenolic compounds in some Indian mango cultivars.* International Journal of Food Sciences & Nutrition, 2004. **55**(2): p. 163-169.
108. Sivakumar, D., Jiang, Y., and Yahia, E.M., *Maintaining mango (Mangifera indica L.) fruit quality during the export chain.* Food Research International, 2011. **44**(5): p. 1254-1263.
109. Mercadante, A.Z. and Rodriguez-Amaya, D.B., *Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango.* Journal of Agricultural and Food Chemistry, 1998. **46**(1): p. 128-130.
110. Rocha Ribeiro, S., et al., *Antioxidant in Mango (Mangifera indica L.) Pulp.* Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 2007. **62**(1): p. 13-17.
111. Abdul Aziz, N.A., et al., *Evaluation of processed green and ripe mango peel and pulp flours (Mangifera indica var. Chokanan) in terms of chemical composition, antioxidant compounds and functional properties.* Journal of the Science of Food and Agriculture, 2012. **92**(3): p. 557-563.
112. Berardini, N., et al., *Utilization of mango peels as a source of pectin and polyphenolics.* Innovative Food Science & Emerging Technologies, 2005. **6**(4): p. 442-452.

113. Dandona, P., Aljada, A., and Bandyopadhyay, A., *Inflammation: the link between insulin resistance, obesity and diabetes.* Trends in Immunology, 2004. **25**(1): p. 4-7.
114. Roongpisuthipong, C., et al., *Postprandial glucose and insulin responses to various tropical fruits of equivalent carbohydrate content in non-insulin-dependent diabetes mellitus.* Diabetes Research and Clinical Practice, 1991. **14**(2): p. 123-131.
115. Contractor, Z., Hussain, F., and Jabbar, A., *Postprandial glucose response to mango, banana and sapota.* The journal of the Pakistan Medical Association, 1999. **49**(9): p. 215-216.
116. Guevarra, M.T.B. and Panlasigui, L.N., *Blood glucose responses of diabetes mellitus type II patients to some local fruits.* Asia Pacific Journal of Clinical Nutrition, 2000. **9**(4): p. 303-308.
117. Fatema, K., et al., *Serum glucose and insulin response to mango and papaya in type 2 diabetic subjects.* Nutrition research (New York, N.Y.), 2003. **23**(1): p. 9-14.
118. Yusof, B.N.M., Talib, R.A., and, Karim, N.A., *A study of blood glucose responses following temperate and tropical fruit ingestion in healthy adults.* Mal J Nutr, 2005. **11**(1): p. 45-57.
119. Reeves, P.G., *Components of the AIN-93 Diets as Improvements in the AIN-76A Diet.* The Journal of Nutrition, 1997. **127**(5): p. 838S-841S.
120. Molnar, J., et al., *Diabetes Induces Endothelial Dysfunction but Does Not Increase Neointimal Formation in High-Fat Diet Fed C57BL/6J Mice.* Circulation Research, 2005. **96**(11): p. 1178-1184.
121. The University of Iowa. *Calculation of AUC using the trapezoidal rule*. 2008; Available from: http://www.uiowa.edu/~c046170/PDFs/auc.PDF.
122. Folch, J., Lees, M., and Sloane Stanley, G.H., *A simple method for the isolation and purification of total lipides from animal tissues.* Journal of Biological Chemistry, 1957. **226**(1): p. 497-509.
123. Mentreddy, S.R., *Medicinal plant species with potential antidiabetic properties.* Journal of the Science of Food and Agriculture, 2007. **87**(5): p. 743-750.
124. Andrikopoulos, S., et al., *Evaluating the glucose tolerance test in mice.* American Journal of Physiology - Endocrinology And Metabolism, 2008. **295**(6): p. E1323-E1332.
125. Kazuyuki M., et al., *Genetic differences among C57BL/6 substrains.* Exp. Anim., 2009. **58**(2): p. 141-149.
126. Freeman, H.C., et al., *Deletion of Nicotinamide Nucleotide Transhydrogenase.* Diabetes, 2006. **55**(7): p. 2153-2156.
127. Nicholson A., et al., *Diet induced obesity in two C57BL/6 substrains with intact or mutant Nicotinamide Nucleotide Transhydrogenase (Nnt) gene.* Obesity (Silver Spring), 2010. **18**(10): p. 1902-1905.
128. Wilkinson, A.S., et al., *Effects of the Mango Components Mangiferin and Quercetin and the Putative Mangiferin Metabolite Norathyriol on the Transactivation of Peroxisome Proliferator-Activated Receptor Isoforms.* Journal of Agricultural and Food Chemistry, 2008. **56**(9): p. 3037-3042.

129. Hayek, T., et al., *Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Apo) A-I. Presentation of a new animal model and mechanistic studies in human Apo A-I transgenic and control mice.* The Journal of Clinical Investigation, 1993. **91**(4): p. 1665-1671.
130. Gutschi, L.M., et al., *Paradoxically Decreased HDL-Cholesterol Levels Associated with Rosiglitazone Therapy.* The Annals of Pharmacotherapy, 2006. **40**(9): p. 1672-1676.
131. Schreyer, S.A., Wilson, D.L., and LeBoeuf, R.C., *C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis.* Atherosclerosis, 1998. **136**(1): p. 17-24.
132. Murase, T., et al., *Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice.* Journal of Lipid Research, 2001. **42**(3): p. 372-378.
133. Bose, M., et al., *The Major Green Tea Polyphenol, (-)-Epigallocatechin-3-Gallate, Inhibits Obesity, Metabolic Syndrome, and Fatty Liver Disease in High-Fat–Fed Mice.* The Journal of Nutrition, 2008. **138**(9): p. 1677-1683.
134. Inoue, M., et al., *Increased expression of PPARγ in high fat diet-induced liver steatosis in mice.* Biochemical and Biophysical Research Communications, 2005. **336**(1): p. 215-222.
135. Nascimento, F.A.M., et al., *Adipose tissue, liver and pancreas structural alterations in C57BL/6 mice fed high-fat-high-sucrose diet supplemented with fish oil (n-3 fatty acid rich oil).* Experimental and Toxicologic Pathology, 2010. **62**(1): p. 17-25.

VITA

SIN EE TER

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF MANGO AND ITS COMBINATION WITH ROSIGLITAZONE ON CLINICAL PARAMETERS IN MICE FED HIGH FAT DIET

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in nutritional sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2012.

Completed the requirements for the Bachelor of Science in dietetics at Iowa State University, Ames, Iowa in May, 2009.

Experience: Employed by Oklahoma State University, Department of the Nutritional Sciences as a graduate research assistant; Oklahoma State University, Stillwater, Oklahoma, 2010-2012.

Professional Memberships: Student member of the American Dietetic Association, student member of the Oklahoma Dietetic Association

Name: SIN EE TER Date of Degree: December, 2012

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE EFFECTS OF MANGO AND ITS COMBINATION WITH ROSIGLITAZONE ON CLINICAL PARAMETERS IN MICE FED HIGH FAT DIET

Pages in Study: 75 Candidate for the Degree of Master of Science

Major Field: Functional food and diabetes

Scope and Method of Study: This study examined the effects of freeze-dried mango (1% or 10% by weight), a fruit rich in vitamins A and C as well as phenolic compounds, in modulating clinical parameters of mice fed high fat (HF) diet. The study also compared the effects of mango to rosiglitazone (50 ppm), a glucose-lowering drug, and determined if addition of mango to a lower dose of rosiglitazone (25 ppm) will have similar effects. Two-month old male C57BL/6 mice were randomly assigned to one of the seven dietary treatment groups (n=8/group) for 60 days: AIN-control (10% fat calories), HF (60% fat calories), HF+1% or 10% mango, HF+50 ppm rosiglitazone, HF+1% mango or 10% mango+25 ppm rosiglitazone. An intraperitoneal glucose tolerance test was performed at 30 and 60 days of treatment. Mice were sacrificed and soft tissues as well as blood were collected after 60 days of treatment. Body composition, clinical parameters (serum HbA1c, plasma glucose, cholesterol, HDL-C, fructosamine, insulin, and triglycerides), and total lipids (i.e. liver and fecal) were determined. Histology was performed on liver and white adipose tissue.

Findings and Conclusions: As expected, body weight increased after the consumption of HF diet. Both doses of mango had similar effect on final body weight to the rosiglitazone group but also not statistically different from the HF group. Mango at 1% dose slightly improved body composition but also not statistically different from the HF group. Plasma free fatty acid was modulated by 1% mango but not quite to the level of rosiglitazone. There were no significant differences in plasma lipids and fructosamine among the HF-fed groups. The HF+50 ppm rosiglitazone group had the lowest glucose area under the curve after a glucose tolerance test. In brief, mango supplementation (1% or 10%) has modest effects on clinical parameters in mice fed high fat diet. Mango combined with a lower dose of rosiglitazone (25 ppm) was not as effective as the higher dose of rosiglitazone (50 ppm) in improving the assessed clinical parameters. Future study should use newly freeze-dried mango powder with known origin and maturity. Additionally, storage conditions should also be carefully controlled to determine the exact relationship between mango supplementation and its effects on clinical parameters.

ADVISER'S APPROVAL: Dr. Edralin A. Lucas