MANGO MODULATES BODY FAT AND PLASMA GLUCOSE AND LIPIDS IN MICE FED HIGH FAT DIET

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CHAPTER I

INTRODUCTION

Obesity has become a major health issue in the United States and the prevalence has doubled in the past 30 years [1]. Parallel to the increase in obesity is a rise in obesity-related chronic diseases such as cardiovascular disease (CVD) and diabetes. CVD continues to be the leading cause of death in the United States and diabetes and obesity are major risk factors of CVD. In 2006, approximately 36.3% of the population in the United States had at least one form of CVD and 33.9% of the population had a body mass index (BMI) over 30 kg/m^2 [2]. The death rate of CVD was 35.3% in 2005 which means 1 out of 3 patients died of CVD [2]. The health cost associated with CVD was estimated to be $475.3 billion in 2008 [2]. The 2006 prevalence of physician-diagnosed prediabetes and diabetes was about 36.5% of the adult population [2]. The total medical cost for diabetes was $174 billion in 2007 [3].

Consumption of a high fat diet and elevated blood glucose and lipids are known contributors to the development of diabetes and CVD [4]. Pharmacological, dietary, and lifestyle modifications such as regular physical activity are ways that can improve blood glucose and lipids. For patients with severe diabetes or hyperlipidemia, medications may be necessary to control the progression of these disorders.

Some of the pharmacological agents used to reduce blood glucose concentration include α-glucosidase inhibitors (e.g. acarbose-precose), biguanide (e.g. metformin), thiazolidinediones (TZDs) (e.g. rosiglitazone), insulin analogs, meglitinides, and d-phenylalanine derivatives [5]. Most of these medications mimic the function of insulin to treat patients with hyperglycemia [5]. Specifically, α-glucosidase inhibitors reduce the absorption of carbohydrates in the small intestine, preventing the elevation of blood [6]. Biguanide, an insulin-sensitizer, increases the uptake of glucose in peripheral tissues and inhibits hepatic gluconeogenesis [6]. Meglitinides and d-phenylalanine enhances insulin secretion while TZDs are similar to biguanide and functions as insulin sensitizers [6]. TZDs are agonists of peroxisome proliferator activated receptor (PPAR) -γ which up-regulates the transcription of genes involved in adipocyte differentiation and production of adipokines such as adiponectin and leptin [6]. TZDs have been shown to effectively decrease plasma glucose and lipid concentrations in insulin-resistant patients [6]. Rosiglitazone is a widely used TZD and a known PPAR-γ agonist commonly used by patients with type 2 diabetes [7]. Although rosiglitazone modulates blood glucose concentrations, it is associated with side effects such as weight gain, anemia, osteoporosis and fluid rentention [8-9].

Peroxisome proliferator activated-receptors (PPARs) are a family nuclear receptor proteins that regulate the expression of genes involved in cellular differentiation and macronutrients metabolism, such as glucose and lipids [10]. PPARs initiate the transcription of genes involved in energy homeostasis and are also key factors in regulating many age-associated pathophysiological diseases related to oxidative stress and inflammatory reaction [10-11]. PPARs can be divided into three subtypes: PPAR-γ,

PPAR-α, and PPAR-β/δ. PPAR-γ is primarily expressed in adipose tissue and is involved in the production of adipocytes and their function [12]. The activation of PPAR-γ improves insulin sensitivity in insulin-sensitive tissues such as the liver and adipose by up-regulating glucose disposal in peripheral tissues and down-regulating glucose production respectively [12]. PPAR-α is crucial for lipid metabolism including β-oxidation, ketogenesis, fatty acid synthesis and lipoprotein metabolism [13]. Fenofibrate is known as a PPAR-α agonist and it has been used to lower cholesterol [14]. The use of fenofibrate is often associated with side effects including skin problems (yellow skin), gastrointestinal effects, muscle pain, and sweating or dizziness [15]. Rhabdomyolysis, a rapid lysis of skeletal muscle tissue due to tissue injury, is also seen often in elderly patients using fenofibrate [16].

Because of the side effects associated with rosiglitazone and fenofibrate, other options are being explored for lowering blood glucose and cholesterol concentrations. Natural food products containing bioactive compounds are continuously being investigated for their potential in reducing chronic conditions such as obesity and diabetes [17-18]. Fruits and vegetables containing phytochemicals have been investigated for their health promoting properties and examples are carrot, dark green leafy vegetables, and apple, bluberry, and mango [19-21]. Mango is a rich source of many bioactive compounds [22]. It is a tropical fruit containing various bioactive components such as polyphenols, carotenoids, and vitamins E and C [23]. A recent study demonstrated that an aqueous extract of mango stem exhibited anti-inflammatory effects by reducing the expression of inflammation-related genes in murine macrophages [24]. Ojewole [25] demonstrated that mango extract effectively lowered blood glucose in

mouse model. The antioxidant and anti-cancer effects of mango juice had been demonstrated by inhibiting free radical production and neoplastic transformation in mammalian cell lines [26]. However, to our knowledge, there are few studies exploring the effect of mango on body composition and glucose and lipid parameters in diet-induced obesity. Therefore, we ***hypothesize*** that mango contains bioactive compounds that can act as PPAR-α and PPAR-γ agonists resulting in modulation of body fat, and plasma glucose and lipid parameters in mice fed high fat diet which aims to induce obesity.

The ***specific aims*** of the study are:

1. To compare the effects of two doses of freeze-dried mango with known PPAR agonists (rosiglitazone and fenofibrate, PPAR-γ and PPAR-α agonist, respectively) in modulating body composition in mice fed high fat diet.
2. To compare the effects of two doses of freeze-dried mango with rosiglitazone and fenofibrate in decreasing the elevation of blood glucose concentrations in mice fed high fat diet.
3. To compare the effects of two doses of freeze-dried mango with rosiglitazone and fenofibrate in reducing blood lipid concentrations in mice fed high fat diet.
4. To begin to explore the mechanism through which mango exerts positive effects on body composition and blood glucose and lipids. Expression of genes involved in glucose and lipid homeostasis will be examined in the liver, white adipose tissue, and skeletal muscle using quantitative Real Time-PCR.

Limitations

There are several ***limitations*** to our study: (1) only a single variety of mango (Tommy/Atkins) was tested and our findings may not represent all mango varieties. It has been shown that varietal differences occur in terms of nutrient and phenolic content [27]; (2) freeze-dried mango was used in the study but fresh mango may have different properties; (3) the dose of freeze-dried mango was based on earlier animal studies using dried fruit such as dried plum, blueberries, or apple for lowering blood cholesterol concentrations [28-30]. The doses (this is not a dose-seeking study), particularly the 10% mango that we used, might not be a reasonable amount for humans. However, our study demonstrated that the 1% dose, a very reasonable dose for humans, is effective in modulating body composition and blood glucose and lipid concentrations in this animal model; (4) the dose and mode of administration of rosiglitazone (50 mg/kg diet) and fenofibrate (500 mg/kg diet) used in the study may not be the most effective in modulating body composition and blood glucose and lipids in this animal model. The dose and mode of administration of rosiglitazone and fenofibrate was based on the findings of Chao et al [31] and Guerre-Millo et al [32], respectively; (5) Because of the use of an animal model, our findings need to be confirmed in humans. A clinical study should be conducted to determine whether the positive results we have observed in this study will translate to the same positive effects in humans.

CHAPTER II

REVIEW OF LITERATURE

Obesity and its associated diseases

Obesity has become one of the most severe epidemics in western societies. The percentage of obese population has doubled in the last decade and the prevalence continues to increase. In the US, the estimated number of overweight or obese adults (age 20 and older) reached 145,000,000 in 2006, representing 66.7% of the adult population [2]. The annual medical cost associated with obesity was $117 billion in 2001, accounting for over 9.1% of the U.S. health expenditure [2]. Overweight and obesity are caused by many factors such as increased caloric dietary intake, consumption of high fat diet foods, lack of physical activity, and heredity. The increased prevalence of obesity has given rise to many comorbid conditions such as diabetes mellitus, insulin resistance, dyslipidemia, hypertension, and atherosclerosis [33].

The World Health Organization (WHO) defines overweight or obesity as "abnormal or excessive fat accumulation that presents a risk to health" [34]. The different stages of overweight or obesity are classified by body mass index (BMI), defined as weight (in kilograms) divided by the square of the height (in meters) ($BMI=weight/height^2$) [34]. The categories of BMI were initially derived from life-

expectancy data collected from insurance companies, and the cut-offs match risks for obesity-related morbidities [35]. BMI equal to or greater than 30 kg/m^2 is defined as "grade 2 obesity" while a BMI of > 25 kg/m^2 is denoted as "overweight" or "grade 1 obesity". The ideal BMI is between 18.5 and 25 kg/m^2. In general when a person's BMI is greater than 25 kg/m^2, he or she is considered overweight [36].

Studies in the past decade have investigated the regulation of obesity and appetite which influences energy homeostasis [37-38]. In general, an abundance of adipose tissue in the body is necessary for survival under critical conditions such as starvation [39]. However, the continuation of excess energy intake when energy expenditure remains stable results in excessive fat storage [40-41]. Obesity can negatively modulate both lipid and glucose metabolism [42-43]. Obesity has been determined to play a key in the regulation of insulin resistance in type 2 diabetes [44].

Adipose tissue is no longer considered only as a site for fat storage but also functions as an endocrine organ [45]. Adipocytes are responsible for secretion of adipokines which contribute to hypertension and dyslipidemia [45]. During insulin resistance, there was an increased adiposity and glucose intolerance seen in mice [46]. Recent studies have also shown that adipose tissue has the ability to secrete proinflammatory molecules such as tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6) and C-reactive protein-1 (CRP-1) [45]. Adipose tissue also secretes many kinds of adipocytokines, such as leptin and adiponectin [47]. Leptin is important in regulating energy balance because it inhibits dietary intake and facilitates energy expenditure [47]. Adiponectin has an inverse relationship with obesity and has been shown to increase insulin sensitivity [47].

Free fatty acids, derived from excessive stored triacylglycerol, play an important role in dyslipidemia and may promote the development hypertriglyceridemia by inhibiting lipogenesis and oxidative stress [33]. In obese subjects, excessive amounts of fat stimulate the release of free fatty acids and promote lipotoxicity inducing oxidative stress [33]. Oxidative stress is produced from lipids and their metabolites, which are not found only in adipose tissue but also in non-adipose tissue such as the liver and pancreas [48-49].

Excess free fatty acids are also associated with insulin-receptor dysfunction leading to insulin resistance, hyperglycemia, and increased hepatic gluconeogenesis. Free fatty acids also inhibit the use of glucose in insulin-stimulated muscle tissue which further exacerbates hyperglycemia [50-51]. Elevated free fatty acids also affect the function of pancreatic β-cell resulting in decreased insulin secretion [52].

Obesity is highly associated with, or is a primary contributing factor to, some of the more severe metabolic diseases. Reducing obesity and overweight have been shown to reduce the total risk and mortality of patients with coronary heart disease [53]. Obesity also has a direct connection with sub-clinical inflammation which is involved in the pathogenesis of many chronic diseases including type 2 diabetes and cardiovascular disease [54].

Treatment of obesity can be generally divided into three categories: lifestyle modifications, medications, or surgical. Lifestyle modifications include changing dietary habits and increasing physical activity. It is well-established that, consuming less calories and increasing energy expenditure is the key to weight loss [55]. Many weight loss diets are aimed at decreasing calorie intake, therefore preventing weight gain. Examples of

weight loss diets include the Mediterranean diet, low-carbohydrate diet, and low-fat diet. The Mediterranean diet is morden nutritional recommended-diet with moderate amounts of fat and is rich in vegetables and limited in red meat [56]. A low-carbohydrate diet, defined as diet programs that restrict cabohydrate intake for purposes of weight loss or treatment of obesity, does not have a strict calorie requirement while low-fat diet is similar to the Mediterranean diet but with lower fat consumption [56]. Engaging in regular physical activity is another aspect of lifestyle modification which increases energy expenditure.

The next treatment of obesity is medications. Weight loss drugs are also being widely used to reduce or prevent overweight and obesity [57]. Sibutramine and Orlistat are two examples of effective weight loss drugs. Sibutramine, in combination with a low-calorie diet, regulates the appetite center which is located in the hypothalamus of the brain causing a reduction in demand for food [58]. Some side effects of sibutramine include back pain, constipation, dry mouth, flu-like symptoms, headache, and nausea [59-60]. Another weight loss medication is Orlistat which blocks the digestion and absorption of dietary fats [61]. Major side effects of orlistat are diarrhea, abdominal pain, hypertension, depression, headache and diabetic ketoacidosis [62].

Bariatric surgery is performed in cases of severe obesity. The major types of bariatric surgery performed are: Roux-en-Y gastric bypass and gastric banding [63]. In Roux-en-Y gastric banding [64], the stomach is dissected into a small pouch with the proximal portion attached to the proximal jejunum, while the distal section of the stomach pouch and a small portion of the jejunum are connected to the distal jejunum. Roux-en-Y gastric bypass restricts food intake and limits nutrient absorption. Gastric

banding is a pure restrictive surgical treatment that divides the stomach into smaller and larger portions by use of prosthetic band. The position of the band in gastric banding can be adjusted to change the volume of proximal pouch [65]. The acute side effects of bariatric surgeries are hemorrhage, obstruction, infection, arrhythmias, and pulmonary emboli. Long-term adverse effects are rhabdomyolysis, neuropathies, internal hernias, and emotional disorders [66].

Due to the side effects of exisiting therapies, the use of nutritional interventions is being widely explored for the treatment of obesity [67].

Diabetes mellitus (DM)

Prevelance

In 2006, there were approximately 57 million adults (25.9%) total in the US diagnosed with prediabetes, which is defined as a condition that occurs when the blood glucose levels are higher than the normal but not high enough to be diagnosed as diabetes [2]. Additionally, 7.7% (approximately 17 million) adults were diagnosed with diabetes in 2006. Type 2 diabetes accounted for 90-95% of the diagnosed diabetes [2]. The mortality due to diabetes in 2005 was about 75,119 and at least 65% of the people with diabetes died of some type of heart disease [2]. Diabetes mellitus is a chronic disease distinguished by abnormally elevated glucose level (hyperglycemia) as a result of insulin resistance. The chronic situation of diabetes can cause lasting dysfunction and failure of various organs, such as the eyes, kidneys, heart, and nerves [68].

Type 2 diabetes mellitus (T2DM)

T2DM is a chronic disease characterized by impaired insulin resistance and insulin

secretion [69]. T2DM occurs when pancreatic β-cells does not produce enough insulin or the body ignores insulin [70]. The impairment of insulin secretion then weakens the insulin response of the body to glucose [70]. Both genetics and environmental factors can affect development of T2DM [71].

Genetics influence important transcription factors such as hepatocyte nuclear factor and insulin promoter factor-1 which control the development and function of the β-cells of the pancreas [72]. About 70-85% of T1DM patients inherit the disease from their parents and the polygenic inheritance associates with environmental factors to develop diabetes [73]. Environmental factors, also known as acquired factors, such as lifestyle, body weight, physical activity, hypertension, dyslipidemia and smoking also play a major role in the development diabetes [71].

Insulin secretion can be influenced by several nutrients such as glucose, some amino acids, and free fatty acids [74-75]. Hormones also affect the secretion of insulin from the pancreas. One of the stimulatory hormones is glucagon-like polypeptide-1, which is a candidate therapeutic agent for T2DM [76]. An example of the inhibitory hormones is somatostatin. Somatostatin is a peptide hormone that inhibits the release of numerous secondary hormones, including insulin [77]. Proinsulin is the precursor of insulin produced from pancreatic β-cells and cleaved into insulin and C-peptide before departing from the β-cells [78]. Even though proinsulin is the precursor of insulin, elevation of proinsulin has been observed in T2DM [78] and may be indicate of insulin resistance [70].

Insulin resistance is defined as “diminished tissue response to insulin at one or more sites in the complex pathways of hormone actio, and is usually heralded by higher

than normal plasma insulin levels, a phenomenon known as compensatory hyperinsulinaemia" [79]. In insulin resistance, plasma insulin levels may remain normal or even elevated but is insufficient to overcome hyperglycemia [73]. Hence, hyperinsulinemia is usually considered as an early stage insulin-resistant marker [80]. Several abnormalities in insulin signaling system may relate to insulin resistance, such as reduced insulin receptor expression and tyrosine kinase activity, which regulates signal transduction for regulating of enzyme activity [81]. The impaired signaling system is a possible explanation for decrease glucose uptake and metabolism in the liver, skeletal muscle, and adipocytes [80, 82-84].

In the liver, insulin is responsible for the inhibition of hepatic glucose formation during postprandial state [85]. When a tissue becomes insulin resistant, both fasting and postprandial glucose production increases, eventually leading to T2DM. Insulin resistance is recognized by a reduction in glucokinase activity and a marked elevation of conversion of substrates to glucose regardless of the presence of insulin [86]. Therefore, glucose is over-produced and under-utilized in diabetics. The increase in free circulating fatty acids seen in type 2 diabetics may be associated with increased hepatic glucose formation since non-esterified fatty acids was found to deteriorate glucose tolenrance in Caucasians [87]. Skeletal muscle also plays an important role in glucose metabolism. The role of the skeletal muscle in glucose homeostasis is as important as in the liver because of the large amount of glucose utilized in the skeletal muscle [84].

GLUT4 is the major glucose transporter present in adipose tissue and skeletal muscle and it is stimulated by insulin [88-89]. In the presence of insulin, GLUT4 is translocated to the cell membrane facilitates glucose uptake. The uptake of glucose from

circulation into peripheral tissues is important in glucose homeostasis since it removes glucose from circulation and then decreases blood glucose level. Defective insulin-induced GLUT4 translocation is often seen in T2DM and may contribute to hyerglycemia [90-91].

Most type 2 diabetics are overweight or obese [68]. A study showed that visceral fat plays a negative role in insulin-resistant patients, and progress of metabolic syndrome was observed after surgical removal of visceral fat [92]. Goodpaster and colleagues [93] demonstrated that losing an appropriate amount of weight by calorie restriction increases insulin sensitivity and reduces total adiposity, and weight loss after one year did improve insulin sensitivity in type 2 dibetics [94].

Prevention and treatment

There are several ways to prevent or delay the development of T2DM. Restricting calorie intake, increasing physical activities and limiting alcohol consumption are ways to prevent or delay the development of T2DM [95]. Once T2DM has developed, treatment options include taking natural products and pharmacological therapy [95].

Calorie restriction, defined as minimized energy intake, but sufficient intake of vitamins, minerals and other important nutrient, also prevents or delays the development of T2DM. Several studies have shown that weight loss can improve insulin sensitivity in skeletal muscle [93, 96-97]. It is also suggested that the surgical removal of visceral fat improved insulin sensitivity in hepatocytes in rodents [98]. As little as a 7% weight loss, type 2 diabetics improved insulin sensitivity by approximately 60% [99]. In other words, weight loss induced by calorie restriction prevents T2DM by promoting insulin sensitity.

Another lifestyle factor that could improve insulin sensitivity is physical activity. Physical activity or exercise is not necessarily accompanied by weight loss; however, improvement in insulin sensitivity was still observed [100]. Possible explanations of improvement of insulin sensitivity are that GLUT4 is elevated during physical activity [101] and an increase in glycogen formation [102]. A weight loss associated physical activity study demonstrated that obesity and insulin resistance were reduced in men who performed exercise and on calorie restriction [97].

Lifestyle modifications may prevent or delay T2DM. These can be effective treatment options. Alcohol consumption is not recommended for type 2 diabetic patients because alcohol has been shown to be related with impaired glucose tolerance [103-106]. A recent study showed a decreased risk of T2DM in elderly women with low intake of alcohol [105]. Furthermore, very low to moderate amount of alcohol consumption has been shown to lower insulin resistance [107]; however the underlying mechanism remains unknown.

Many studies have shown that ~ 1 in 3 adults with T2DM use therapies such as acupuncture, chiropractic care, yoga, and herbal remedies to prevent and/or treat type 2 DM [108-110]. In addition to these interventions, consumption of supplements such as chromium, garlic, ginseng and various herbal or botanical supplements, are other ways of preventing and/or treating T2DM [111]. Taking these supplements for the prevention and/or treatment of diabetes remains controversial. For example, the ability of chromium supplements to improve insulin sensitivity yields conflicting results and needs to be further examined [112]. Various therapies and consumption of supplements are being widely used; however pharmacological therapy is more concerned in clinical treatment of

T2DM.

Pharmacological therapy provides by far the most effective option in the treatment of T2DM. Metformin is an anti-diabetic drug that has been widely used to treat T2DM, particularly in overweight or obese diabetics [113]. Type 2 diabetics have a higher rate of glucose production than non-diabetics and metformin can effectively lower this rate by over 30% [114]. Metformin reduces glucose production by inhibiting hepatic gluconeogenesis [115]. Physiologically, metformin activates AMP-activated protein kinase (AMPK) which suppresses gluconeogenesis in the liver [116]. A recent study illustrated that activated AMPK could promote the expression of small heterodimer partner (SHP), a transcriptional factor that inhibits the activity of two enzymes (glucose-6-phosphatase and fructose 1, 6-bisphosphatase) in the hepatic gluconeogenesis [117]. Other mechanisms by which metformin enhance insulin sensitivity includes increasing glucose uptake and promoting β-oxidation is rodent skeletal muscle [118].

Thiazolidines (TZDs), such as pioglitazone and rosiglitazone, are group of medications that have been commonly used in T2DM. TZDs activate PPAR-γ, resulting in the transcription of insulin-sensitive genes (examples are GLUT4 and aP2) involved in lipid and glucose metabolism in the liver, adipose tissue, and skeletal muscle [119-120]. Pioglitazone improves fasting plasma glucose and HbA_{1c}, and increases insulin sensitivity and plasma adiponectin in T2DM subjects [121]. Pioglitazone decreases insulin resistance in the liver and peripheral tissues which can promote glucose uptake. However, heart failure is a major side effect associated with the use of TZDs [122].The role of rosiglitazone in reducing glucose will be discussed in a later section.

Pramlintide is an analog of human amylin, a pancreatic islet cell hormone released

from β-cells along with insulin [123]. Administration of pramlintide in type 2 diabetics significantly improved postprandial hyperglycemia [123-125]. Additionally, pramlintide is reported to exhibit a weight loss property [126] possibly thru delaying gastric emptying [127]. Similar to amylin, pramlintide enhances insulin sensitivity by regulating glucose metabolism thru modulating postpradial glucose levels [123], inhibiting glucagon secretion [128], and delaying gastric emptying [129].

Exenatide acts as a potent agonist of glucagon-like peptide-1. Exenatide enhances β-cell proliferation in rodents but this proliferation is not exhibited in humans [130]. Similar to glucagon-like peptide-1, exenatide enchances glucose-dependent insulin secretion by the pancreatic β-cells, inhibits glucagon secretion and decreases the both rate of gastric emptying and food intake, although the mechanism of action is still under investigation [131-133]. A two-year clinical study showed exenatide to significantly reduce plasma HbA1c by 1% and promote weight loss [124].

Peroxisome proliferator activated receptors (PPARs) and their physiological roles

Peroxisome proliferator activated receptors (PPARs) belong to the super family of steroid nuclear receptor of transcription factors that modulate ligand-dependent activation and suppression of genes [134]. The binding of PPARs to specific peroxisome proliferator response elements (PPREs) allows them to mediate transcription for genes involved in lipid and glucose metabolism [135]. This property of PPARs gives them potential therapeutic applications as a treatment for lipid and glucose associated metabolic syndrome.

Structurally, PPAR proteins contain six domains: A/B, C, D, E and F domains. The

A/B domain is considered the activation site for transcription. The C domain is where DNA binds to the location of zinc finger. The D domain is referred to as the DNA sequence recognition site, and E and F domains are the sites for ligand binding [136]. The DNA-binding domain primarily recognizes and binds to specific response element [42]. PPARs form heterodimers with attachment of retinoic acid X receptor (RXR) and binds to PPREs in target genes [137]. Once PPARs have been activated by ligand, they are ready to bind to specific promoting regions that stimulate the transcription of RNA polymerase of target genes [137].

Figure 1. Six domains of PPAR protein [134]:

Three types of PPARs that have been identified: PPAR-α, PPAR-β/δ, and PPAR-γ [138]. PPAR-α is most abundantly expressed in the liver, brown adipose tissue, kidney, heart and skeletal muscle; PPAR β/δ is expressed in the intestine, adipose tissue, skeletal muscle, heart and brain and PPAR-γ is expressed in adipose tissue and the intestine [137-138]. The ligands for PPARs are divided by their target proteins, for example PPAR-α ligands can be divided into natural and synthetic groups [139]. Natural ligands for PPAR-α include fatty acids and eicosanoids while synthetic ligands for PPAR-α are fibrates such as fenofibrate and bezafibrate, and nonsteroidal anti-inflammatory medications, such as prostaglandins. Natural ligands of PPAR-γ are fatty acid derivatives such as arachidonic acid metabolites [140]. Synthetic ligands of PPAR-γ are TZDs and nonsteroidal anti-inflammatory chemicals, such as indomethacin and ibuprofen [141]. Prostacylin, a natural compound produced by vascular endothelial and smooth muscle

cells when stimulated by cytokines, is a ligand of PPAR-β/δ [142].

Transcription initiated by PPARs is responsible for the synthesis of proteins that are involved in various biological pathways [143]. PPAR-α spares glucose during the fasting state and promotes fatty acid oxidation and ketone body formation. PPAR-γ regulates lipid storage and glucose utilization in a fed state [12]. PPAR-β/δ is a regulator that enhances fatty acid catabolism in skeletal muscle [144]. The anti-inflammatory effects of PPAR-α and PPAR-γ are well established based on *in vitro* and *in vivo* studies [145]. For example, PPAR-α and PPAR-γ agonists appear to delay the initiation phase of the inflammatory process in paw edema model of inflammation [146]. Because the focus of our study is on PPAR-α and -γ, hence, PPAR-β/δ will not be discussed further.

PPAR-α is a direct regulator of genes that function in fatty acid uptake and oxidation in the liver [147]. PPAR-α null mice become dyslipidemic because peroxisomal and mitochondrial fatty acid metabolizing enzymes cannot metabolize long-chain fatty acids [148-149]. Interestingly PPAR-α null mice do not develop high-fat diet-induced insulin resistance, but show signs of fat accumulation [150].

PPAR-γ ligands have been used to increase insulin sensitivity in T2DM [134]. The most common PPAR-γ ligands are the TZDs which include troglitazone, pioglitazone, and rosiglitazone [151]. Genes that are regulated by PPAR-γ are involved in maintaining lipid and glucose homeostasis [134]. PPAR-γ regulates glucose homeostasis by modulating hormones secreted from adipocytes [143, 152]. Thus, PPAR-γ is an insulin sensitizing factor and regulates of the expression of adipokines, such as increasing adiponectin and decreasing leptin, resistin, and tumor necrosis factor-α (TNF-α) [152].

More specifically, PPAR-γ target genes are effective in adipocyte hormones and influencing the release of free fatty acid from adipocytes.

Insulin signaling is suppressed by elevation of free fatty acids in the skeletal muscle and liver, thus insulin resistance might also be a consequence of free fatty acid metabolism. The differentiation of preadipocytes into adipocytes requires PPAR-γ as a transcriptional factor. Treatment with TZDs causes a decline in abdominal fat and an increase in subcutaneous fat in diabetic patients [151]. The insulin sensitizing property of PPAR-γ is believed to be achieved thru the activation of fatty acid transporters (FATP1 and CD36) and glycerol kinase. The activation of the above proteins can cause retention of fatty acids in the adipose tissue. A clinical study showed that a dominant mutation of PPAR-γ resulted in a syndrome of critical hyperinsulinemia and early stage of hypertension [153].

The functions of the target genes of PPAR-α and PPAR-γ are discussed below. Lipoprotein lipase (LPL) promotes clearance of lipid from both chylomicrons and VLDL for storage as triacylglycerol in adipose tissue. Acetyl-CoA carboxylase (ACC-α) is an isoform of acetyl CoA carboxylase which is the regulatory enzyme in fatty acid synthesis. Acyl CoA oxidase (ACOX1) utilized molecular oxygen and produces hydrogen peroxide in the first step of lipid oxidation in the liver [154]. Fatty acid transport protein (FATP) is a transporter protein located in the plasma membrane that imports free fatty acids into hepatocytes [155]. Medium-chain acyl-CoA dehydrogenase (MCAD) is the enzyme which initiates breakdown of acyl-CoA into acetyl-CoA during β-oxidation in the mitochondria [155]. Hormone sensitive lipoprotein lipase (HSL) hydrolyzes triacylglycerol to monoacylglycerol in fatty acid oxidation. The translocation of glucose

transport protein 4 (GLUT4) from adipocyte cytosol to the plasma membrane is stimulated by insulin and GLUT4 is responsible for the transport of glucose into the cytosol. TNF-α is secreted by adipocytes and is increased in obesity, known to contribute to insulin resistance [154]. Adipocyte protein 2 (aP2), also called fatty acid binding protein, is a known PPAR-γ target gene which is mostly expressed in white adipose tissue [156]. Adiponectin is a plasma protein primarily secreted from adipose tissue which is responsible for regulating glucose and lipid homeostasis [157].

The detail of PPAR-α and PPAR-γ agonists are being discussed in the following section.

PPARs agonists-fenofibrate and rosiglitazone

Fenofibrate

Fenofibrate, an isopropyl ester of 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanoic acid, was first identified by Thorp and Waring in 1962 [158]. It has a chemical formula of $C_{20}H_{21}O_4Cl$ and a molecular weight of 306.83 Da [159]. Fenofibrate is insoluble in water but quickly converts to the water soluble and pharmacologically active form, fenofibric acid, following oral administration [160].

Fenofibrate as well as its fibric acid derivatives influence plasma lipoprotein levels by regulating the synthesis and clearance of lipoproteins. It can affect many pathways involved in lipid metabolism, cholesterol esterification, platelet aggregation, and platelet-derived growth factor (PDGF) [161]. The intake of fenofibrate causes several side effects in patients. The most common ones are skin problems, gastrointestinal

effects, muscle pain, and sweating or dizziness [15]; especially rhabdomyolysis is seen in elderly patients [16]. Although an isolated incidence, fenofibrate has been reported to increase myopathy [162]. Moreover, combination of fenofibrate with statin has been shown to cause rhabdomyolysis [163].

Fenofibrate and its metabolite fenofibric acid are ligands for PPAR-α. Fenofibrate reduces plasma triglyceride concentration by up-regulating the expression of genes involved in β-oxidation. Other potential benefits of fenofibrate are decreasing platelet aggregation and the level of plasma fibrinogen [164]. The mechanism for fenofibrate-induced reduction in total cholesterol and LDL is not quite clear. In human studies, treatment with fenofibrate or its fibric acid derivatives significantly reduced the level of LDL, VLDL and total cholesterol due to suppression of cholesterol synthesis [165-168]. Fenofibrate had been shown to inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a rate-limiting enzyme involved in cholesterol synthesis and clearance [169]. Moreover, fenofibrate have been shown to increase the formation of bile from cholesterol, leading to intracellular cholesterol reduction and LDL receptor generation in the liver [170].

Fibric acid derivatives are also known to raise plasma HDL due to rapid generation of the HDL apolipoproteins A-I and A-II. Fenofibrate also promotes the clearance of apo AI; however, the increase in generation exceeds clearance, and thus there is an overall increase in apo A-II. Activity of lecithin-cholesterol acyltranferase (lCAT), an enzyme that functions on the exterior of HDL particle that converts intravascular free cholesterol to cholesteryl ester, was shown to be enhanced by fenofibrate resulting to an increase in plasma HDL levels [170].

A 15-year clinical trial on fenofibrate was done in 1980s and over 3,500 patients participated in the research [171]. Oral administration of fenofibrate for one month resulted in a decrease in plasma triglyceride by 30-60%. A similar reduction of triglyceride concentrations was observed in a study by Tesone and colleagues [172]. In patients with type IIb hyperlipoproteinemia (i.e. elevated LDL and VLDL), fenofibrate reduced triglycerides by approximately 30% and total plasma cholesterol by about 20% [170]. The recommended dosage of fenofibrate is 300 mg/day. A reduction of LDL cholesterol was observed in patients with type IIa hyperlipoproteinemia being treated with fenofibrate [173]. In clinical trials, the continuous intake of fenofibrate (300mg/day) causes decline in total and LDL cholesterol and elevation in HDL concentration [174].

An animal study by Rodney and colleagues [175] showed that fenofibrate upregulates lipoprotein lipase resulting to an increase in triglyceride clearance in the liver and other tissues. The upregulation of lipoprotein lipase also decreases the production of triglyceride in the liver resulting in a decrease of the release of substrate from adipose tissue [175]. Fenofibrate was also shown to reduce plasma triglyceride concentration by approximately 40% in mice fed fructose diet [176]. Another animal study showed that fenofibrate decreased plasma triglyceride levels but increased LDL [177].

Rosiglitazone

Rosiglitazone [(±)-5-[[4-[2-(methyl-2-pyridinylamino)-ethoxylphenyl] methyl]-2, 4-thiazolidinedione, (Z)-2-butenedioate (1:1)] is a PPAR-γ agonist belonging to the TZD family [178].

The mechanism of action of rosiglitazone is not related to sulfonylureas, biguanides, or α-glucosidase inhibitors. Rosiglitazone is a PPAR-γ agonist, which acts mostly on adipose tissue [7]. Rosiglitazone binds with higher affinity to PPAR-γ compared to other TZDs [179]. The ligand dependent activation of PPAR-γ induces gene expression involved in adipogenesis, insulin signaling, and glucose transport. Therefore activation of PPAR-γ has the effect of reducing insulin resistance in target tissues [180].

There are several ways by which rosiglitazone contributes to the treatment of T2DM. Rosiglitazone enhances insulin sensitization in target tissues without promoting the pancreatic secretion of insulin [181]. GLUT4 is a target gene of PPAR-γ and its production and translocation take place whenever PPAR-γ is activated, since GLUT4 is an insulin-dependent transporter. Under healthy conditions, insulin promotes the translocation of GLUT4 to the cell surface to facilitate glucose uptake [179]. If GLUT4 expression is diminished at the cell surface, glucose uptake is reduced and it is considered the primary cause of the development of insulin resistance [182]. PPAR-γ activation may also decrease hepatic glucose production and may promote insulin-dependent glucose uptake in adipose tissue and skeletal muscle [179]. Rosiglitazone stimulates the differentiation of adipocytes and suppresses the release of fatty acids from adipose tissue [183].

Rosiglitazone decreases the expression of lipoprotein lipase and adipocyte lipid-binding protein, enzymes involved in fatty acid metabolism in the adipose tissue [184-185]. Rosiglitazone has also the potential to reduce the expression of leptin *in vivo* and *in vitro*, an adipokine controlling appetite and energy expenditure [186].

Rosiglitazone can also indirectly promote glucose uptake by the skeletal muscle thru improving insulin sensitivity [187]. Activated PPAR-γ stimulates adipocytes to signal the skeletal muscle to increase insulin sensitivity. Both *in vivo* and *in vitro* evidences indicate that TNF-α participates in this signaling pathway since elevation of TNF-α induces insulin resistance in adipose tissue [188]. Rosiglitazone suppresses the inhibitory effects of TNF-α on insulin activity [183, 189].

In humans, rosiglitazone improves insulin sensitivity and secretion, glucose tolerance, and adipocytokines in T2DM patients [190]. T2DM patients receiving rosiglitazone for three months exhibited significant reductions in $HbA_{1C,}$ fasting plasma glucose, fasting free fatty acid, mean glucose GTT, and plasma TNF-α. Also, there was a significant increase in insulin sensitivity despite increases in body weight. These results demonstrate that rosiglitazone improves insulin sensitivity and secretion, and the circulating level of plasma adipokines [190]. Another clinical study showed that T2DM patients treated with rosiglitazone exhibited a 9-14% drop in LDL level and a 5-15% elevation in HDL concentrations [191].

Because of the side effects associated with current pharmacotherapy, nutrition therapies are being explored for lowering blood glucose and cholesterol concentrations. Natural food products containing bioactive compounds are continuously being investigated for their potential in reducing chronic conditions such as obesity and diabetes [17-18].

Health benefits of mango

For this section, studies on mango were divided according to the part of mango used for the study (pulp, peel, kernel, plant) or how it is prepared, such as stem bark extraction or seed extraction.

Mango flesh or pulp

Mango (*Mangifera indica* L.) is a tropical fruit relatively rich in various nutrients and phytochemicals such as polyphenols, vitamin C and dietary fiber. Mango flesh or pulp is the only edible part of the fruit. The mango flesh contains polyphenols, terpenoids, carotenoids, fatty acids [23, 192] and other trace elements such as calcium, vitamin A and C [193]. Over 180 compounds have been identified in at least 20 cultivars of mango. Total concentration of these compounds is about 18-123 mg/kg the fresh fruit [194]. The typical composition of mango pulp is reported in **Table 1**.

Ribeiro and colleagues [27] investigated the pulp composition in four varieties of mango (Haden, Tommy Atkins, Palmer and Uba) and three potential antioxidant substances (total phenolics, carotenoids, and ascorbic acid). The variety with each phytochemical is listed in **Table 2**. Clearly there are varietal differences in terms of nutrient and antioxidant content of mango [27].

The first study on mango flesh was conducted by Frylinck and Dubery [195]. In this experiment, a calcium-dependent protein kinase was purified from the extract of ripening mango fruit tissue [195]. This isolated protein phosphorylates histone III-S to a lesser extent of casein kinase phosphorylating substrates on serine or threonine residues. This particular protein kinase may be an important enzyme in the ripening process of the

mango fruit because ripening is known to involve increased turnover of membrane inositol phospholipids which, in turn, is often coupled to increases in intracellular calcium ion [195].

Table 1. Typical nutrient composition of mango pulp [196]

Nutrient	Units	Value per 100 grams
Protein	g	0.51
Total lipid (fat)	g	0.27
Carbohydrate (by difference)	g	17.00
Total dietary fiber	g	1.8
Total sugars	g	14.80
Calcium	mg	10
Potassium	mg	156
Phosphorus	mg	11
Magnesium	mg	9
Vitamin C (total ascorbic acid)	mg	27.7
Total folate	mg	14
Vitamin A	IU	765
β-carotene	mcg	445
Vitamin E	mg	1.12

Table 2.The pulp compositin in four mango cultivars

	Haden	**Uba**	**Palmer**	**Tommy Atkins**
Total phenolic (mg/100g)	48.4	208.7		
Total carotenoid (mg/100g)	1.91		2.63	
β-carotene (μg/100g)		2220	661.27	
Total ascorbic acid (mg/100g)		77.71		9.79

The anticancer and antioxidant effects of mango juice and juice extract have also been reported [26]. The antioxidant property of mango juice and juice extract was assessed using three standard methods: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), and Folin's assay. The anti-cancer property was evaluated by determining the effect on cell cycle and neoplastic transformation of mammalian BALB/c 3T3 cells [26]. The results of antioxidant property of mango juice and juice extract obtained from three standard methods were consistent with each other. Treatment with mango juice and juice extracts showed an inhibitory effect on the G0/G1 phase of cell cycle progression. In neoplastic transformation assay, whole mango juice

significantly reduced amount of transformed foci, indicating the suppressive effect of mango juice exerts on tumor cells [26].

A study by Prasad and coworkers [197] determined the hepatoprotective effects of mango using Swiss albino mice given 7-12-dimethylbenzanthracene (DMBA). After treatment with lupeol (an effective inhibitor in laboratory models of prostate and skin cancers) and mango pulp extract, DMBA-induced oxidative stress was improved, as indicated by the restored antioxidant enzyme activities and reduced lipid peroxidation. They also found that lupeol and mango pulp extract effectively reduced apoptosis in the mouse liver [197].

A similar study [198] explored the antioxidant effect of lupeol and mango pulp extract in response to androgen-induced oxidative stress using Swiss albino mice. Animals received oral treatment of either lupeol or mango pulp extract along with testosterone injection for 15 days. At the end of the study, prostate samples were collected and reactive oxygen species (ROS), lipid peroxidation, and activity of antioxidant enzymes were assessed. Supplementations with lupeol and mango pulp extract each reduced ROS and lipid peroxidation and restored abundance of antioxidant enzymes [198]. The results demonstrated a fact the mango pulp can be used as an antioxidant agent.

Mango peel

Mango peel is one of the inedible parts of mango and a major by-product in pulp manufacturing. Some bioactive compounds have been characterized from mango peel

including 5-alkyl-and 5-alkenylresorcinols [199]. These bioactive molecules are believed to have potential pharmacological effects that relate back to their use as traditional medicine [200].

5-(11’ Z-heptadecenyl) - and 5(8’Z, 11’Z-heptadecadienyl)-rescorcinols were also purified from mango peel and they can potentially inhibit the activity of cyclooxygenase COX-1 and COX-2, known pro-inflammatory mediators [199]. Furthermore, a study demonstrated that mango peel inhibited hydrogen peroxide-induced hemolysis in a dose dependent manner [193]. The mango peel extract also prevented membrane protein from degradation induced by hydrogen peroxide. These findings supported that mango peel contains compounds which can protect erythrocytes against oxidative stress [193].

Mango kernel or seed

The mango kernel has also been utilized in research [201-202]. Mango kernel contains twenty-one gallotannins (15.5mg/g) which may contribute to its antioxidative, immunomodulatory, analgesic, and anti-inflammatory properties [203]. In an immune response study, the findings showed that mango kernel treatment effectively improved superoxide anion production, serum protein, albumin, and survivability in fish treated with dried mango kernel compared to the control group [202], and the resutls demonstrated that mango kernel enhanced immune response and allowed fish to be more resistant to pathogen infection. In addition, methanolic and aqueous extracts of mango seed have been shown to have potential anti-diarrheal activity in diabetic mice [201].

Mango stem bark extract

Several phenolic components have been purified from mango stem bark [204]. The major phenolic compounds isolated from the stem bark include mangiferin, gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, (+)-catechin, (-)-epicatechin, and benzoic acid and benzoic acid propyl ester [204]. Vimang-® is the brand name of aqueous extract of *Mangifera indica* family stem bark and mangiferin is the major polyphenol present in Vimang [205].

Anti-diabetic and hypolipidemic effect of stem bark extract

To our knowledge, very few studies have been conducted on glucose lowering and hypolipidemic effects of mango. Aderibigbe and colleagues [206] determined the glucose lowering effects of mango extract using a streptozotocin (STZ)-induced diabetic mice model. Their findings showed that mango aqueous stem bark extract significantly reduced blood glucose in STZ-induced diabetic mice compared to the hypoglycemic drug chlopropanid. Another study conducted by the same group indicated that aqueous extract of mango leaves has also been shown to prevent glucose-induced hyperglycemia in STZ-induced diabetic rats [206].

Another *in vivo* study demonstrated that daily oral treatment with one of the two doses of mangiferin (10 and 20 mg/kg) for 28 days had an anti-diabetic effect and atherogenic potential in STZ-induced diabetes in rats. Significant reductions in plasma glucose concentration due to mangiferin were observed in this animal model. Furthermore, mangiferin exhibited a hypolipidemic effect as shown by significant reductions in plasma total cholesterol, triglycerides, and LDL concentrations along with

an increase of HDL-cholesterol. The findings of this study provide evidence of the anti-diabetic and hypolipidemic properties of mangiferin [207].

In an *in vivo* study [208], *Mangifera indica* bark extract exhibited the highest percentage inhibition of α-glucosidase compared with *Swertia chirata* whole plant and *Lawsonia inermis* leave extract. *Swertia chirata* is in the gentianaceae family and commonly seen as clearing nut tree and *Lawsonia inermis* is a flowering plant named Henna that has been traditionally used for body stain. The inhibitory effect of *Mangifera indica* bark extract on α-glucosidase may explain the role of mango in preventing obesity and diabetes.

Anti-inflammatory effect of stem bark extract

The anti-inflammatory properties of mango extract have also been explored. Garrido and colleagues [209] conducted a study on the analgesic and anti-inflammatory effects of Vimang-®. Vimang-® at a dose of 50-1000 mg/kg significantly inhibited formalin-induced pain (an indicator of analgesic function) and edema (an indicator of anti-inflammatory function) in mice. The same group [210] tested the anti-inflammatory activity of Vimang both *in vivo* and *in vitro.* The *in vivo* study demonstrated that arachidonic acid and phorbol myristate acetate-induced ear edema was reduced after application of Vimang [210]. Vimang also reduced serum levels of TNF-α in this animal model of inflammation. Vimang inhibited the induction of PGE_2 in stimulated macrophage cells (RAW 264.7) [210].

García and colleagues [211] investigated the effect of Vimang on specific mouse antibody generation induced by inoculation of parasites in mice spleen cells. Vimang but

not mangiferin, significantly decreased antibody production from the third week after inoculation and the inhibitory activity reached a peak at fourth week after inoculation. Vimang also significantly suppressed the production of IgG but not IgM, which suggestes that the primary antibody against antigens is not affected by Vimang. Mangiferin had no effect on neither IgM nor IgG2a, but significantly improved production of IgG1 and IgG2b. These results suggested that mango stem bark extracts may play a role in modulating the humoral response in mouse spleen cells.

Vimang and mangiferin have also been shown to suppress the expression of COX-2 and nitric oxide synthase in Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats [212]. Vimang (0.5-0.1 mg/ml) and mangiferin (0.025 mg/ml) inhibited nitric oxide synthase expression (smooth muscle) more in SHR than in WKY rats whereas COX-2 was expressed more in WKY than in SHR. Their findings demonstrate that certain doses of both Vimang and mangiferin suppressed the expression of nitric oxide synthase and COX-2 indicating the anti-inflammatory property of Vimang.

Ojewole [25] used a powder form of stem bark extract of *Mangifera indica* to examine its potential analgesic, anti-inflammatory, and antidiabetic effects in laboratory animals. Mice were used to investigate the analgesic effects while rats were used for assessing the anti-inflammatory and anti-diabetic properties. Significant dose-dependent analgesic effects were observed in mice given 50-800 mg/kg of stem bark extract compared to mice fed morphine (analgesic reference). The same dose of the extract significantly inhibited inflammatory-induced paw edema and lowered glucose level in rats.

The effect of Vimang on eosinophil migration in an animal model of asthma has also been investigated [213]. Eosinophil migration into the bronchoalveolar space was decreased by Vimang treatment (50 mg/kg for 18 days) of *Toxocara canis*-infected BALB/c mice. Along with the reduction of eosinophil migration, reduction of IL-5 in serum and eotaxin in the lungs was observed and therefore prevented animal from inflammation. Moreover, Vimang treatment was determined non-toxic to laboratory animals since normal body weights were maintained during infection. The findings of this study demonstrated the anti-inflammatory effect of Vimang and its potential use in the treatment of eosinophilic disorders, such as eosinophilic esophagitis, eosinophilic gastritis and eosinophilic colitis [213].

Mangiferin along with other major phenolic compounds, catechin and epicatechin, were shown to effectively attenuate human T lymphocytes activation-induced cell death by downregulating the intracellular levels of reactive oxygen species (ROS) and calcium [214]. Activation-induced cell death was required to maintain peripheral lymphocyte homeostasis and is induced by ROS with calcium influx into the cytosol [214].

Antioxidant effect

A compound purified from stem bark extract of *Mangifera indica* L., QF808, was shown to modulate protein and hepatic microsome peroxidation [215]. QF808 reduced oxidation and lipid peroxidation in bovine serum albumin (BSA), but the activity of NADPH-dependent cytochrome P-450 reductase was not affected. These results suggested that QF808 have potent antioxidant activity possibly acting as a free radical

scavenger in lipid peroxidation.

Stem bark extract of *Mangifera indica L.* also protected T lymphocytes against activation-induced cell death in an *in vitro* study [216]. The stem bark extract attenuated the accumulation of reactive oxygen species and intracellular Ca^{2+}, factors required in the expression of CD95L which plays a role in AIDS. Furthermore, stem bark extract also inhibited activation-induced cell death which is stimulated by T cell receptor and triggers an intracellular signaling pathway that promotes the formation of ROS and the increase in cystolic Ca^{2+}. Therefore, stem bark extract allows the enhancement of T-cell survival [216].

Another *in vitro* study by Rodriguez and colleagues [217] compared the effects of mangiferin with epigallocatechin gallate (EGCG) on energy metabolism and malondialdehyde formation, an indicator of oxidative stress. The study found that treatment with Vimang, mangiferin, and EGCG significantly increased the resistance to oxygen species production induced by hydrogen peroxide in human erythrocytes [217]. Hydrogen peroxide-initiated energy charge potential was restored with treatments in a dose-dependent manner. The treatments also increased the level of energy metabolism related compounds that were depleted by hydrogen peroxide damage such as ATP, GTP and total nucleotides [217].

The antioxidant effect of Vimang was further investigated using an *in vivo* study [218]. Vimang inhibited degradation of 2-deoxyribose mediated by Fe (III)-EDTA plus ascorbate. This result illustrated that Vimang dose not simply remove $OH^{\cdot}$ radicals to inhibit 2-deoxyribose degradation, but acts as an anti-oxidant by complexing iron ions [218]. Similarly, Andreu [219] demonstrated that Vimang exhibit iron-complexing

property and protect the liver mitochondria against lipoperoxidation induced by Fe^{2+} -citrate. Vimang (equivalent to 10 μM mangiferin) protected rat liver mitochondria against mitochondrial swelling induced by Fe^{+2}-citrate [219]. The stem bark extract also functioned as a free radical scavenger to protect the mitochondria from lipoperoxidation, confirming the potential antioxidant effect of Vimang [219].

Sánchez and colleagues [220] investigated the effect of Vimang on hepatic ischemia/reperfusion-associated injury. Female Wistar rats with hepatic ischemia received an oral treatment of Vimang (50, 110, and 250 mg/kg of body weight) for seven days. Ischemia/reperfusion-induced transaminase elevation and DNA fragmentation were reduced in a dose-dependent manner by Vimang. These findings suggested that Vimang could be used as a natural treatment for preventing oxidative damage induced by hepatic injury.

An *in vivo* study demonstrated the effect of Vimang against oxidative stress cytotoxicity in rat hepatocytes [221]. Male rats fed diet containing different concentrations of Vimang (20, 50 and 100 μg/ml) had lower glucose-glucose oxidase induced ROS formation in hepatocytes. Hepatocyte cytotoxicity and lipid peroxidation were both suppressed by Vimang in a dose- and time-dependent manner. In addition, Vimang also inhibited superoxide radical formation [221].

In isoproterenol-induced myocardial infarcted rats, the enzyme activities involved in the tricarboxylic acid cycle and antioxidant defense system were inhibited and lipid peroxidation was promoted in the heart mitochodria [222]. In mangiferin-pretreated rats, fatty acid oxidation was improved and enzyme activities were restored in isoproterenol-induced myocardial infarcted rats [222]. The activities of serum lactate dehydrogenase

and creatine phosphokinase isoenzymes were elevated in isoproperenol-treated rats, indicating myocardial damage. Treatment with mangiferin significantly elevated the levels of heart tissue enzymatic antioxidants and serum non-enzymatic antioxidants compared to isoproterenol-treated rats. These results suggest a protective effect of mangiferin against isoproterenol-induced myocardial infarction [222].

A recent *in vivo* study demonstrated the ability of Vimang or mangiferin in preventing oxidative stress in murine mitochondria of LDL receptor knockout mice [223]. The LDL receptor knockout mice were more sensitive to oxidative stress and mitochondrial membrane permeability transition, increasing its risk of developing atherosclerosis. Oral supplementation with Vimang or mangiferin altered the membrane permeability transition to control levels in LDLr-null liver mitochondria. Vimang or mangiferin also significantly decreased the production of ROS in both LDLr-null liver mitochondria and spleen lymphocytes. Vimang and mangiferin restored both the antioxidant capacity and organelle oxidation-reduction homeostasis in the liver mitochondria of atherosclerosis-prone mice.

Another *in vivo* study demonstrated the anti-inflammatory and antioxidant effects of the stem bark aqueous extract of *Mangifera indica* [224]. Stem bark extract inhibited early and late stage T cell activation, as well as the development the S-phase of cell cycle and proliferation induced by T cell receptor [224]. TNF-α-induced IκBα degradation and the binding of NF-κB and DNA are also inhibited by stem bark extract [224].

The protective effect of Vimang against oxidative stress was also studied in elderly humans [225]. Elderly subjects (>65 years) received 900 mg of mango stem bark extract daily for 60 days. The plasma lipid peroxides and oxidized glutathione (GSSG),

serum peroxidation potential, extracellular superoxide dismutase activity and total antioxidants were measured at baseline and 15, 30, and 60 days after treatment. Vimang significantly increased the activity of extracellular superoxide dismutase and total antioxidants in elderly participants in comparison to young subjects (<26 years). Vimang also decreased the level of GSSG in elderly subjects. All the above observations suggest that Vimang is effective in preventing age-related oxidative stress in humans [225].

Anti-allergic effect

The anti-helmintic and anti-allergic properties of Vimang and mangiferin in laboratory mice were investigated by García and colleagues [226]. Vimang (500 mg/body weight/day) or mangiferin (50 mg/body weight/day) was given to mice infected with *Trichinella spiralis* throughout the parasite life cycle. Oral treatments of both Vimang and mangiferin significantly decreased the number of parasite larvae in the musculature, but did not influenced adult parasites in the gut. Results also suggest that treatment with Vimang or mangiferin significantly reduced serum level of anti-*Trichinella* IgE which plays an important role in the pathogenesis of allergy.

The anti-allergic effects of Vimang were also demonstrated in female Balb/c mice with inflammatory allergy [227]. At the beginning of the study, mice were immunized by intraperitoneal injection of 100 μg of ovoalbumin (OVA). Mice received the same amount of OVA a week after first immunization. Twenty-one days after first immunization, Vimang at concentration of 50, 100 or 250 mg/kg and 50 mg/kg of mangiferin was given to the mice. The primary finding of the study was that both Vimang

and mangiferin decreased IgE serum levels in OVA-immunized mice, suggesting an anti-allergic effect [227].

Radioprotective effect

The radioprotective effect of mangiferin was demonstrated using DBAxC57BL mice [228]. Mice received different dosages of mangiferin one hour prior to the radiation exposure. Results indicated that mangiferin treatment decreased the symptoms of radiation sickness and mortality compared to the control group. The radioprotective effect of mangiferin increased in a dose-dependent manner and reached peak at a concentration of 2 mg/kg body weight.

Cytotoxic effect

The cytotoxic effects as well as possible interactions of Vimang with P450 enzymes and how it affects GSH and lipid peroxidation were examined in rat hepatocytes [229]. No cytotoxic effects were detected 24 hours after Vimang (up to 1000 μg/ml) treatment and a moderate cytotoxicity was monitored 48 hours to 72 hours after exposure at concentration of 500 and 1000 μg/ml. Vimang at concentration above 100 μg/ml acts as a modulator for P450 enzymes. GSH protein and lipid peroxidation were significantly inhibited after a 36 hours pretreatment with Vimang at concentration of 25-200 μg/ml.

The cytotoxic effect of mangiferin was examined in rat hepatocytes by Rodeiro and colleagues [230]. A moderate cytotoxic effect was detected after 72 hours of treatment with mangiferin. Moreover, mangiferin decreased the activity of P450 enzymes

in a concentration dependent manner after 48 hours exposure, indicating interaction mangiferin with metabolism [230].

Other protective effects of stem bark extract

Mangiferin also exhibited a gastroprotective effect against gastric injury induced by ethanol and indomethacin in rats [231]. The protective effects of mangiferin on gastric damage were determined by alterations in mean gastric lesion area. Mangiferin at concentrations of 3, 10 and 30 mg/kg body weight decreased ethanol-induced gastric damage by 30, 35, and 63%, and indomethacin-induced by 22, 23, and 57%, respectively. In addition, mangiferin successfully prevented the depletion of gastric mucosal non-protein sulfhydryl content in mice, suggesting an antioxidative effect. This study provided evidence to support the protection of mangiferin against gastric injury caused by ethanol and indomethacin, and a possible underlying mechanism might be mangiferin's antioxidative action.

In a more recent study, the effect of mango components (i.e. mangiferin and quercetin, and a metabolite of mangiferin, norathyriol) on PPARs was examined [232]. It is reported that quercetin as well as norathyriol exhibited an inhibitory effect on activation on all three subtypes of PPARs. However, mangiferin did not suppress the transactivation of any PPARs. This study suggests that components of mango may modify the transcription of PPARs and could promote health benefits [232].

Vimang or mangiferin also exhibited an effect on preventing iron overload injury in rat model [233]. Rats received three dosages of Vimang or 40 mg/kg body weight of mangiferin for seven days before and after administration of 100 mg/kg of iron-dextran.

Their results demonstrated that Vimang or mangiferin prevented iron overload in plasma as well as oxidative stress in hepatocytes and reduce the percentage of saturation and iron content in the liver. Moreover, Vimang or mangiferin enhanced serum iron-binding capacity and decreased the number of abnormal Kupffer cells in iron-loaded hepatic tissue. The authors suggested that Vimang or mangiferin are potential antioxidants and promote liver iron excretion to protect liver against iron overload [233].

The effect of mango stem bark extract on the contraction of the smooth muscle of the trachea of rats was investigated by Agbonon and colleagues [234]. After inducing a substantial contraction in the smooth muscle of the trachea of male Wistar rats by acetylcholine, the trachea was treated with mango stem bark extract (1, 2 and 4 mg/ml) [234]. Stem bark extract of mango dose-dependently relaxed the isolated trachea in this model, indicating possible use of mango in the treatment of asthma.

By now, there are many studies conducted on the nutritional benefits of mango stem bark extract. However, there is very limited source focusing on the mango flesh which is the only edible part of mango. Therefore, we conducted this study to illustrate the nutritional benefits of consuming mango flesh in maintaining individual's health.

CHAPTER III

METHODOLOGY

Animal care and dietary treatments

Fifty two, three-month old C57BL/6J male mice were purchased from Harlan Teklad (Indianapolis, IN). Animal handling and procedures were approved by Institutional Animal Care and Use Committee at Oklahoma State University. Mice were acclimated for three days and were fed with standardized powdered rodent diet (AIN 93M) [235]. After acclimation, mice were weighed and randomly divided into six dietary treatment groups (n=8 mice/ group): (1) AIN-93M [235]; (2) high fat diet (based on formulation of [236]); (3) high fat diet with fenofibrate (0.05mg/kg diet, Cayman Chemicals, Ann Arbor, Michigan); (4) high fat diet with rosiglitazone (0.005mg/kg diet, Cayman Chemicals, Ann Arbor, MI); (5) high fat diet with 1% (w/w) freeze dried mango (low dose); and (6) high fat diet with 10% (w/w) freeze dried mango (high dose). Tommy Atkins variety mango was freeze dried, ground, analyzed for its nutrient composition (**Table 3**) and incorporated into the diet at 1% or 10% concentration by weight. All high fat diets were adjusted to have the same macronutrient composition, as well as calcium and phosphorus (**Table 4)**. Mice were given food and deionized water *ad libitum* and were weighed weekly.

Glucose tolerance test

After eight weeks of dietary treatment, a glucose tolerance test was performed (n=6 mice/ group). Mice were fasted overnight and 50 μl of blood from the tail was used to determine baseline blood glucose concentrations using a home glucose testing kit (Onetouch Ultra, LifeScan, Inc. Milpitas, CA). Mice were then injected intraperitoneally with 20% glucose solution (2 g/kg body weight). Blood glucose concentration was again determined at 5, 15, 30, 60 and 120 minutes post glucose injection. Area under the curve was calculated by the trapezoidal rule [237].

Necropsy and tissue processing

After two months of dietary treatment, mice were weighed and injected with ketamine/xylazine cocktail (80 and 8 mg/kg body weight, respectively). Body composition was determined using a GE Lunar Piximus (Fitchburg, WI). Blood was collected from carotid artery into EDTA coated tubes. Plasma was obtained by centrifugation of whole blood for 20 minutes at 1306 g and stored at -80°C until further analyses. White adipose tissue, liver, spleen, kidney and skeletal muscle were collected. White adipose tissue, liver and kidney were weighed, snap-frozen and stored at -80°C. Skeletal muscle was snap-frozen and stored at -80°C.

Clinical analyses

Alfa Wassermann (West Caldwell, NJ) clinical chemistry analyzer was used to determine plasma concentrations of glucose, non-esterified fatty acids (NEFA), total cholesterol, HDL-C, and triglycerides. Kits were purchased from Alfa Wasserman (West

Caldwell, NJ) except for NEFA (Wako Diagnostics, Richmond, VA) and the manufacturer's instructions were strictly followed.

Plasma glucose concentrations were determined based on its reaction with adenosine triphosphate (ATP) in the presence of hexokinase and magnesium which produces glucose-6 phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate and NAD^+ to form 6-phosphogluconate and NADH. The NADH produced, which strongly absorbed at 340nm, is proportional to the amount of glucose in the plasma (Alfa Wasserman, West Caldwell, NJ).

To determine total cholesterol, esterified cholesterol was first hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids. Endogenous free cholesterol and cholesterol released from cholesterol esters are oxidized by cholesterol oxidase to produce hydrogen peroxide (H_2O_2). Peroxidase then catalyzes the reaction between H_2O_2, p-hydroxybenzoic acid and 4-aminoantipyrine to produce a red-color quinoneimine complex which strongly absorbs at 505 nm. The amount of chromogen generated is proportional to the total cholesterol concentration when measuring the absorbance at 505 nm/647 nm (Alfa Wasserman, West Caldwell, NJ).

The HDL-cholesterol assay uses a unique reagent which solubilizes only the HDL lipoproteins and also inhibits reaction of the cholesterol enzymes with LDL, VLDL and chylomicron lipoproteins. HDL cholesterol is then released from lipoproteins and reacts with cholesterol esterase and oxidase which in the presence of a chromogen produces color for detection. Chromogen formation is determined by measuring the absorbance bichromatically at 592/692 nm which is proportional to the HDL cholesterol concentration (Alfa Wasserman, West Caldwell, NJ).

Plasma triglycerides are hydrolyzed by lipase to release glycerol and free fatty acids. In the presence of ATP and glycerol kinase, glycerol is phosphorylated to glycerol-1-phosphate and ATP is converted to ADP. Glycerol-1-phoaphate is then oxidized by glycerol phosphate oxidase to produce H_2O_2. H_2O_2 reacts with *p*-chlorophenol and 4-aminoantipyrine to produce a red-color quinoneimine, a reaction is catalyzed by peroxidase. Chromogen formation is determined by measuring the absorbance bichromatically at 505/692 nm which is proportional to the triglyceride concentration (Alfa Wasserman, West Caldwell, NJ).

In the presence of ATP and CoA, NEFA is converted to thiol esters of CoA, when treated with acyl-CoA synthetase. Then acyl-CoA is oxidized to produce H_2O_2 in the presence of acyl-CoA oxidase and peroxidase. This conversion allows the condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline with 4-aminoantipyrine to create a purple-color product which absorbs strongly at 550 nm (Wako Diagnostics, Richmond, VA).

Plasma leptin, adiponectin and insulin

Enzyme linked immunosorbent assay (ELISA) kits from Linco Research (St. Charles, MO) were used to determine plasma concentrations of leptin, adiponectin and insulin. The wells of microtiter plates were coated with anti-mouse adiponectin, leptin or insulin monoclonal antibodies before use. The plate was then coated with a second biotinylated anti-mouse polyclonal antibody to capture the analytes in the samples. Unbound materials from samples were then washed off and horseradish peroxidase was applied on to the immobilized biotinylated antibodies. The activity of horseradish

peroxidase was monitored using 3, 3', 5, 5'-tetramethylbenzidine as a substrate and absorbance at 450 nm was measured using a microplate reader (Biotek Synergy, Winooski, VT). The captured mouse adiponectin, leptin or insulin in the plasma was directly proportional to the increase in absorbance at 450nm/590nm (Linco Research, St. Charles, MO).

RNA isolation

RNA was extracted from the liver, white adipose tissue, and skeletal muscle using STAT60 (TEL-TEST INC, TX), chloroform (Sigma, St Louis, MO) and isopropyl alcohol (Sigma, St Louis, MO). All the tissue samples were homogenized in STAT-60 (1ml of STAT-60/50-100 mg tissue). The tubes were left at room temperature for 5 minutes followed by addition of chloroform (0.2ml chloroform /ml STAT-60). Tubes were centrifuged at 11,750 g for 15 minutes at 4°C and the top aqueous layer was transferred to a new tube. Isopropyl alcohol (0.5ml/ml STAT-60) was added to each tube and was inverted thoroughly. Tubes were left for 10 minutes at room temperature and then centrifuged at 11,750 g for 10 minutes at 4°C. The supernatant was discarded and 75% ethanol (1ml ethanol/1 ml STAT-60) was added to each tube, vortexed, and centrifuged again at 4592 g for 5 minutes at 4°C. The supernatant was discarded and the precipitated RNA was air-dried for 2-3 minutes and then dissolved in 100 µl DEPC H_2O. The concentrations and quality of the RNA samples were determined by NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE). The quality of RNA was confirmed with agarose gel electrophoresis. RNA samples were stored at -80 °C until

used for RT-PCR. All qRT PCR results were evaluated by the comparative cycle number at threshold (C_T) method using cyclo or 36b4 as the invariant control.

Gene expression by real time-PCR

Genes involved in lipid metabolism, PPAR-α, LPL, ACC-α, ACOX1, FATP and MCAD were assessed in the liver. Cyclophilin was used as housekeeping gene.

For white adipose tissue, genes involved in glucose and lipid metabolism were assessed. These genes include HSL, GLUT4, and ACOX1, ACC-α, LPL, and PPAR-γ, aP2, and adiponectin. Cyclophilin was used as housekeeping gene.

For skeletal muscle, genes involved in both glucose and lipid metabolism (GLUT4, ACOX1, ACCα, FATP and MCAD) were assessed and used 36b4 as housekeeping gene. The functions of these genes were discussed in the previous section.

Amplifications were performed in a 10 μL reaction mix containing unkown cDNA samples on a 384-well plate, forward and reverse primers, and SYBR Green I PCR reagents (Applied Biosystems, Foster City, CA, USA). The primers used for PCR are shown in **Table 5**. Amplifications of cDNA were detected constantly by realtime quantitative PCR on 7900 HT Fast Real-time PCR system (Applied Biosystems, Foster City, CA). The PCR amplification protocol is as follows: (i) initial attenuation at 95°C for 10 minutes; and (ii) three-segment amplification and quantification involving 40 cycles of 50°C for 120 s, 95°C for 10 min, 95°C for 9 s, 60°C for 60 s, 95°C for 9 s, and 60°C for 9 s.

Statistical analyses

Statistical analyses involved computation of least square means and standard error (SE) for each of the treatment groups using SAS version 9.1 (SAS Institute, Cary, NC). Analysis of variance and least square means were calculated using the general linear model procedure and the means were compared using Fisher's least significant difference for comparing groups. Differences were considered significant at $P < 0.05$.

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

Nutrients	**Amount *(g/100 g)***
Protein	3.94
Fat	6.46
Fiber	3.84
Ash	2.19
Calcium	0.0424
Phosphorus	0.0941
Carbohydrate	74.8
Calories *(kcal/100g)*	373

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

Table 4: Composition of the experimental diets

Ingredient	Normal diet[a] (AIN-93M)	High fat diet[b,c]	High fat diet + 1% mango	High fat diet + 10% mango
amount (g/kg diet)				
Mango	0	0	10	100
Total carbohydrate	721	370	370	370
Cornstarch	621	100	94	25.2
Sucrose	100	270	270	270
Contribution of mango			7.5	74.8
Total fat	40	350	350	350
Soybean Oil	40	40	39.4	33.5
Lard	0	310	310	310
Contribution of mango			0.646	6.47
Total Protein	140	180	180	180
Casein	140	180	179.6	176.1
Contribution of mango			0.394	3.9
Total fiber	50	50	50	50
Cellulose	50	50	49.6	31
Contribution of mango			0.384	3.83
Vitamin Mix (AIN 93VX)	10	10	10	10
Mineral Mix (AIN 93 MX)	35	35	35	35
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 diet[e]	379	549	553	546

[a]Based on AIN-93M formulations containing 72% carbohydrate, 4% fat and 14% protein by calories (Reeves et al., 1997).
[b]High fat diet (37 % carbohydrate, 35% fat and 18% protein by calories) based on formulation of Molnar et al (2005).
[c]Rosiglitazone and fenofibrate were from Cayman Chemicals (Ann Arbor, MI) and added to the high fat diet at a dose of 50 and 500 mg/kg diet, respectively.
[e]Analyzed by NP Analytical Laboratories (St. Louis, MO)

Table 5: Primers used for real-time PCR

Gene	**Forward sequence (F)** **Reverse sequence (R)**
Peroxisome proliferator-activated receptor alpha (PPARα)	(F) 5`-CGTACGGCAATGGCTTTATC (R) 5`-AACGGCTTCCTCAGGTTCTT
Peroxisome proliferator-activated receptor gamma (PPARγ)	(F) 5`-CCCACCAACTTCGGAATCA (R) 5`-TGCGAGTGGTCTTCCATCAC
Lipoprotein lipase (LPL)	(F) 5`-GGACTGAGAATGGCAAGCAA (R) 5`-CCACTGTGCCGTACAGAGAAA
Hormone sensitive lipoprotein lipase (hsLPL)	(F) 5'-GGAGCACTACAAACGCAACGA (R) 5'-TCGGCCACCGGTAAAGAG
Acyl-Coenzyme A oxidase 1 (ACOX-1)	(F) 5'-AGATTGGTAGAAATTGCTGCAAAA (R) 5'-ACGCCACTTCCTTGCTCTTC
Acetyl-CoA carboxylase alpha (ACCα)	(F) 5'-GGCAGCTCTGGAGGTGTATG (R) 5'-TCCTTAAGCTGGCGGTGTT
Glucose transporter 4 (GLUT 4)	(F) 5`-CCTTTCTCATTGGCATCATTTC (R) 5`-CACGGCCAAGACATTGTTG
Adiponectin	(F) 5`-TCACGGTGTACATGAAAGATGTG (R) 5`-GAGAACGGCCTTGTCCTTCT
Tumor necrosis factor alpha (TNFα)	(F) 5`-CTGAGGTCAATCTGCCCAAGTAC (R) 5`-CTTCACAGAGCAATGACTCCAAAG
Fatty acid transporter protein 1 (FATP1)	(F) 5'-CCGTATCCTCACGCATGTGT (R) 5'-CTCCATCGTGTCCTCATTGAC
Fatty acid transporter protein 5 (FATP5)	(F) 5'-GACCACTGGACTCCCAAAGC (R) 5'-GACAGCACGTTGCTCACTTGT
Medium-chain acyl dehydrogenase (MCAD)	(F) 5'-GATGCCATCACCCTCGTGTAAC (R) 5'-AAGCCCTTTTCCCCTGAAG
adipocyte protein 2 (aP2)	(F) 5`-GCCAAGCCCAACATGATCA (R) 5`-TTCCACGCCCAGTTTGAAG
36B4	(F) 5'-CACTGGTCTAGGACCCGAGAAG (R) 5'-GGTGCCTCTGAAGATTTTCG
Cyclophilin (cyclo)	(F) 5`-TGGAGAGCACCAAGACAGACA (R) 5`-TGCCGGAGTCGACAATGAT

CHAPTER IV

FINDINGS

Food intake, body and tissue weights

Two mango groups had similar daily food intake compare to fenofibrate, rosiglitazone and high fat diet groups. As mice were fed *ad libitum*, the average food intake of the mice receiving the normal diet was significantly greater than those receiving the high fat (**Table 6**). Because of the higher food intake, caloric intake of the mice fed normal diet was significantly higher than all the other treatment groups (**Table 6**).

After eight weeks of feeding, there were no significant differences in the initial and final body weights (**Table 6**) as well as weekly body weights among groups (**Table 7**). Despite similar body weight, adipose tissue weight was highest in the group fed high fat diet (**Table 6**). The two doses of mango, similar to fenofibrate and rosiglitazone, prevent the increase of adipose tissue to that of the normal diet (**Table 6**). Consumption of fenofibrate resulted to a significantly heavier liver, an effect not seen in the low dose mango (**Table 6**). There were no significant differences in kidney and spleen weights (**Table 6**).

Whole body composition

There was no significant difference in lean mass among all groups (**Table 8**). The two doses of mango, similar to rosiglitazone and fenofibrate, significantly reduced fat mass (**Table 8**) and percent body fat to the level of those receiving normal diet (**Figure 2**). As expected, high fat group had the highest fat mass (**Table 8**) and percent body fat (**Figure 2**).

Glucose tolerance test

Mice fed with the normal diet tended (P=0.0544) to have the highest baseline glucose concentration while those in the rosiglitazone group the lowest (**Table 9**). No significant differences in blood glucose concentration were observed after 5 and 15 minutes of glucose injection. However, significant differences were observed after 30, 60, and 120 minutes after glucose injection (**Table 9**). Mango supplementation at 1% dose is the most effective in normalizing blood glucose concentrations after a glucose tolerance test as indicated by comparing the area under the curve (**Figure 3**). High fat diet had the highest blood glucose concentration at these time points while 1% mango had the lowest (**Table 9**). Rosiglitazone, a known hypoglycemic agent, fenofibrate and 10% mango is similar to the high fat diet in terms of its effect on area under the curve (**Figure 3**).

Clinical chemistry parameters

There were significant differences in plasma glucose concentration after two months of dietary treatment (**Figure 4**). Mice fed with 1% mango had similar plasma

concentration to rosiglitazone group, which was significantly lower than the normal diet, high fat diet, and fenofibrate (**Figure 4**). Mango supplementation at 10% dose had an intermediate effect on plasma glucose concentration (**Figure 4**).

There were significant differences in plasma total cholesterol among the groups after two months of dietary treatment (**Figure 5**). Mice receiving the normal diet and rosiglitazone had the lowest plasma total cholesterol concentrations (**Figure 5**). Interestingly, mice receiving fenofibrate had the highest total cholesterol and those receiving high fat diet had an intermediate total cholesterol values (**Figure 5**). Plasma total cholesterol concentrations of mice in the low or high dose mango was similar to the rosiglitazone and the normal diet group (**Figure 5**).

There were also significant differences in plasma HDL-C concentrations among the treatment groups (**Table 10**). Mice receiving rosiglitazone or the two doses of mango had similar HDL-C to those in the normal diet (**Table 10).** Mice receiving fenofibrate had the highest HDL-C (**Table 10)**.

All other treatment groups reduced NEFA to the level of the normal diet group except for the high dose mango which had an in-between effect (**Figure 6**). Non-esterified fatty acid (NEFA) was highest in the high fat group (**Figure 6**).

There was no significant difference in plasma triglycerides concentrations among any of the treatment groups (**Table 10**).

Plasma leptin, adiponectin and insulin

Significant differences in plasma leptin concentrations were observed after two months of dietary treatment (**Figure 7**). Mice in the normal and high fat diet had similar

plasma leptin concentrations which was significantly higher than the mice receiving mango (**Figure 7**). Rosiglitazone and fenofibrate had an intermediate effect on plasma leptin concentrations (**Figure 7**).

Mice in the high fat diet, fenofibrate and 1% mango had similar plasma adiponectin concentrations which was significantly higher than the mice in the rosiglitazone group (**Figure 8**). Mice receiving the high dose of mango had similar plasma of adiponectin concentrations to the mice in normal diet and rosiglitazone group (**Figure 8**).

There was no significant difference in plasma insulin concentrations among the treatment groups (**Table 10**).

Gene expression by real time PCR

There were significant differences in the liver gene expression (relative mRNA) of enzymes involved in fatty acid synthesis, ACCα and β oxidation ACOX1 and MCAD after two months of dietary treatment (**Figures 9-14**). Low dose mango has similar relative ACCα mRNA to that of normal and high fat diet (**Figure 9**). Relative to the normal diet, fenofibrate supplementation had significantly higher gene expression of ACCα (**Figure 9**). High dose mango had the lowest gene expression of ACCα followed by rosiglitazone (**Figure 9**). Similar pattern was observed in the gene expression of ACOX1 (**Figure 10**) and MCAD (**Figure 11**) in the liver.

After two months of dietary treatment, there was a significant difference in the liver gene expression of the transport protein FATP5 (**Figure 12).** High fat and fenofibrate groups had significantly higher liver FATP5 gene expression compared to the

rosiglitazone and mango groups (**Figure 12**). Gene expression of transport protein FATP1 in the skeletal muscle was not affected by dietary treatment (**Table 10**).

Only fenofibrate increased gene expression of liver LPL relative to the normal diet group (**Figure 13)**. Normal diet, high fat diet, and fenofibrate treatment had significantly higher liver relative mRNA of PPARα than the low and high dose mango groups (**Figure 14**).

There were no significant differences in the relative mRNA level of genes examined in the white adipose tissue (ACCα, ACOX1, adiponectin, aP2, GLUT4, hsLPL, LPL and PPAR-γ) and in the skeletal muscle (ACOX1, FATP1, GLUT4, LPL, and MCAD) (**Table 11**).

Table 6: Effects of mango, rosiglitazone, and fenofibrate on food intake and body and tissue weights of mice fed high fat diet for two months[1]

Para-meters	AIN-93M	High Fat	High Fat + 1% Mango	High Fat + 10% Mango	High Fat + Feno-fibrate	High Fat + Rosi-glitazone	P value
Food intake *(g/d)*	4.9 ± 0.1^{a}	2.4 ± 0.1^{b}	2.4 ± 0.1^{b}	2.6 ± 0.1^{b}	2.4 ± 0.1^{b}	2.6 ± 0.1^{b}	<0.0001
Calories (*kcal/d*)	18.5 ± 0.3^{a}	13.3 ± 0.3^{bc}	13.5 ± 0.3^{bc}	14.0 ± 0.3^{b}	13.0 ± 0.3^{c}	14.2 ± 0.3^{b}	<0.0001
Body weights (g)							
Initial	23.4 ± 0.5	23.3 ± 0.5	23.3 ± 0.6	23.8 ± 0.6	23.0 ± 0.5	23.1 ± 0.6	0.9342
Final	29.4 ± 1.0	30.9 ± 1.0	27.7 ± 1.2	29.1 ± 1.1	28.0 ± 1.0	26.8 ± 1.1	0.1136
Tissue weights (mg)							
Adipose tissue	830 ± 164^{b}	1530 ± 164^{a}	730 ± 186^{b}	870 ± 174^{b}	828 ± 164^{b}	524 ± 174^{b}	0.0037
Liver	994 ± 46^{bc}	909 ± 46^{c}	897 ± 52^{c}	1058 ± 49^{b}	1644 ± 46^{a}	923 ± 49^{bc}	<0.0001
Spleen	70 ± 14	63 ± 14	84 ± 16	100 ± 15	66 ± 14	99 ± 15	0.2848
Kidney	361 ± 11	330 ± 11	343 ± 12	358 ± 12	338 ± 11	341 ± 12	0.3343

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

Table 7: Effects of mango, rosiglitazone, and fenofibrate on weekly body weights of mice fed high fat diet for two months[1]

	AIN-93M	**High fat**	**High fat+1% mango**	**High fat+10% mango**	**High fat+ Feno-fibrate**	**High fat+ Rosigli-tazone**	**P value**
	(grams)						
Initial weight	23.4 ± 0.5	23.3 ± 0.5	23.3 ± 0.6	23.8 ± 0.6	23.0 ± 0.5	23.1 ± 0.6	0.9342
Week 1	23.5 ± 0.6	24.7 ± 0.6	24.6 ± 0.7	24.4 ± 0.7	23.8 ± 0.6	23.4 ± 0.7	0.5920
Week 2	23.4 ± 0.7	24.6 ± 0.7	25.7 ± 0.8	25.3 ± 0.8	24.7 ± 0.7	23.4 ± 0.8	0.2173
Week 3	26.8 ± 0.8	26.1 ± 0.8	27.3 ± 1.0	27.5 ± 0.9	26.1 ± 0.8	25.3 ± 0.9	0.5114
Week 4	27.8 ± 0.9	28.3 ± 0.9	29.0 ± 1.0	29.4 ± 0.9	27.1 ± 0.9	26.9 ± 0.9	0.3496
Week 5	28.5 ± 1.1	29.8 ± 1.1	29.7 ± 1.2	29.4 ± 1.1	27.8 ± 1.1	28.1 ± 1.1	0.6814
Week 6	29.9 ± 1.2	32.4 ± 1.2	31.0 ± 1.3	31.0 ± 1.2	28.9 ± 1.2	29.3 ± 1.2	0.3175
Week 7	31.4 ± 1.2	33.1 ± 1.2	31.2 ± 1.4	31.7 ± 1.3	29.6 ± 1.2	29.3 ± 1.3	0.2993
Final weight	29.4 ± 1.0	30.9 ± 1.0	27.7 ± 1.2	29.1 ± 1.1	28.0 ± 1.0	26.8 ± 1.1	0.1136

[1]Values are mean ± SE, n=8/group.

Table 8: Effects of mango, rosiglitazone, and fenofibrate on lean and fat mass of mice fed high fat diet for two months[1]

Parameters	AIN-93M	High Fat	High Fat + 1% Mango	High Fat + 10% Mango	High Fat + Feno-fibrate	High Fat + Rosi-glitazone	P value
Lean mass *(g)*	20.8 ± 0.5	19.6 ± 0.5	21.1 ± 0.5	20.4 ± 0.5	20.2 ± 0.5	20.5 ± 0.5	0.4847
Fat mass *(g)*	7.2 ± 1.0^{b}	10.1 ± 1.0^{a}	5.4 ± 1.0^{b}	6.8 ± 1.0^{b}	6.5 ± 1.0^{b}	5.7 ± 1.0^{b}	0.0255

[1]Values are mean ± SE, n=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 mango, rosiglitazone, and fenofibrate on blood glucose during a glucose tolerance test of mice fed high fat diet for two months[1]

	AIN-93M	**High fat**	**High fat +1% mango**	**High fat +10% mango**	**High fat +Feno-fibrate**	**High fat + Rosigli-tazone**	**P value**
	(mg/dl)						
Baseline	153.7±11.1	120.7 ± 11.1	112.3 ± 12.0	127.3 ± 12.0	128.1 ± 11.1	102.1 ± 11.1	0.0544
5 min	262.0 ± 21.4	234.0 ± 21.4	259.3 ± 23.2	233.8 ± 23.2	247.7 ± 21.4	178.3 ± 21.4	0.1025
15 min	431.4 ± 29.0	407.3 ± 29.0	382.0 ± 31.3	352.2 ± 31.3	417.0 ± 29.0	377.4 ± 29.0	0.4623
30 min	389.6 ± 30.7^{bc}	490.0 ± 30.7^{a}	349.8 ± 33.2^{c}	420.8 ± 33.2^{abc}	467.3 ± 30.7^{ab}	431.7 ± 30.7^{abc}	0.0441
60 min	319.9 ± 34.5^{ab}	413.3 ± 34.5^{a}	216.8 ± 37.3^{b}	351.5 ± 37.3^{a}	373.6 ± 34.5^{a}	323.9 ± 34.5^{a}	0.0137
120 min	171.0 ± 21.9^{ab}	217.3 ± 21.9^{a}	114.7 ± 23.7^{b}	232.7 ± 23.7^{a}	167.6 ± 21.9^{ab}	188.3 ± 21.9^{a}	0.0177

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

Table 10: Effects of mango, rosiglitazone and fenofibrate on plasma triglycerides, HDL-cholesterol, and insulin concentrations of mice fed high fat diet for two months[1]

Para-meters	AIN-93M	High Fat	High Fat + 1% Mango	High Fat + 10% Mango	High Fat + Feno-fibrate	High Fat + Rosi-glitazone	P value
TG *(mg/dl)*	53.2 ± 6.8	52.1 ± 6.8	58.4 ± 7.7	38.4 ± 7.7	36.1 ± 6.8	46.5 ± 7.2	0.2146
HDL *(mg/dl)*	37.0 ± 4.1^{c}	52.9 ± 3.9^{b}	41.2 ± 4.8^{bc}	44.1 ± 4.4^{bc}	75.6 ± 3.9^{a}	33.7 ± 4.4^{c}	<0.0001
Insulin *(ng/ml)*	0.19 ± 0.02	0.23 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.02	0.2674

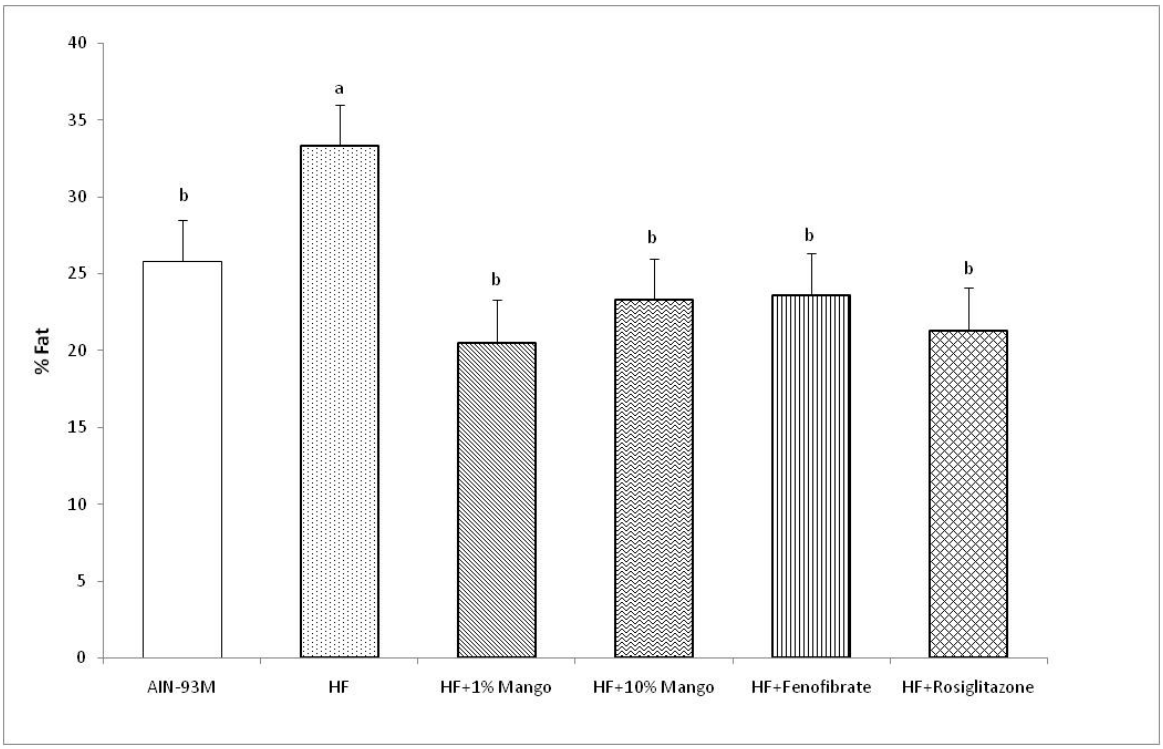
[1]Values are mean ± SE, n=8/group; within a row, values that do not share the same letters are significantly (P<0.05) different from each other. TG=triglycerides, HDL=high density lipoprotein cholesterol.

Table 11: Effects of mango, rosiglitazone, and fenofibrate on gene expression (relative mRNA) in white adipose tissue and skeletal muscle of mice fed high fat diet for two months[1]

Para-meters	AIN-93M	High Fat	High Fat + 1% Mango	High Fat + 10% Mango	High Fat + Feno-fibrate	High Fat + Rosi-glitazone	P value
White adipose tissue (Control gene=Cyclo)							
ACCα Mean C_T =21.0	1.00 ± 5.2	1.16 ± 5.2	0.99 ± 4.8	11.92 ± 4.8	2.19 ± 4.8	1.35 ± 4.8	0.5431
ACOX1 Mean C_T =21.0	1.00 ± 0.5	0.49 ± 0.5	1.19 ± 0.5	1.07 ± 0.5	2.04 ± 0.5	0.93 ± 0.5	0.4379
Adipo-Nectin Mean C_T =20.4	1.00 ± 0.3	0.50 ± 0.23	0.80 ± 0.3	0.92 ± 0.3	0.71 ± 0.3	1.31 ± 0.3	0.4306
aP2 Mean C_T =20.4	1.00 ± 0.3	0.58 ± 0.3	0.82 ± 0.3	1.04 ± 0.3	0.64 ± 0.3	1.80 ± 0.3	0.0598
GUT4 Mean C_T =20.7	1.00 ± 0.3	0.59 ± 0.3	0.73 ± 0.3	0.50 ± 0.3	0.83 ± 0.3	1.00 ± 0.3	0.6595
hsLPL Mean C_T =21.0	1.00 ± 3.4	0.87 ± 3.4	1.42 ± 3.1	7.82 ± 3.1	2.25 ± 3.1	2.68± 3.1	0.6334
LPL Mean C_T =20.7	1.00 ± 0.3	0.59 ± 0.3	125 ± 0.3	0.61 ± 0. 3	1.01 ± 0.3	1.37 ± 0.3	0.3315
PPARγ Mean C_T =20.7	1.00 ± 0.3	0.55 ± 0.3	1.15 ± 0.3	0.59 ± 0.3	0.78 ± 0.3	0.83 ± 0.3	0.6666
Skeletal muscle (Control gene=36b4)							
ACOX1 Mean C_T =23.2	1.00 ± 0.1	1.02 ± 0.1	0.9 ± 0.2	1.17± 0.2	0.78 ± 0.1	1.15 ± 0.1	0.4660
FATP1 Mean C_T =23.7	1.00 ± 0.9	2.70 ± 0.8	1.25 ± 0.9	2.33 ± 0.9	1.22 ± 0.8	2.08 ± 0.8	0.6317
GLUT4 Mean C_T =20.6	1.00 ± 0.1	0.81 ± 0.1	0.98 ± 0.1	0.63 ± 0.1	0.62 ± 0.1	0.63 ± 0.2	0.1516
LPL Mean C_T =23.2	1.00 ± 0.1	0.88 ± 0.1	0.83 ± 0.2	0.96 ± 0.2	0.73 ± 0.1	0.79 ± 0.1	0.8073
MCAD Mean C_T =23.2	1.00 ± 0.1	1.32 ± 0.1	1.04 ± 0.1	1.00 ± 0.1	0.93 ± 0.1	0.88 ± 0.1	0.2229

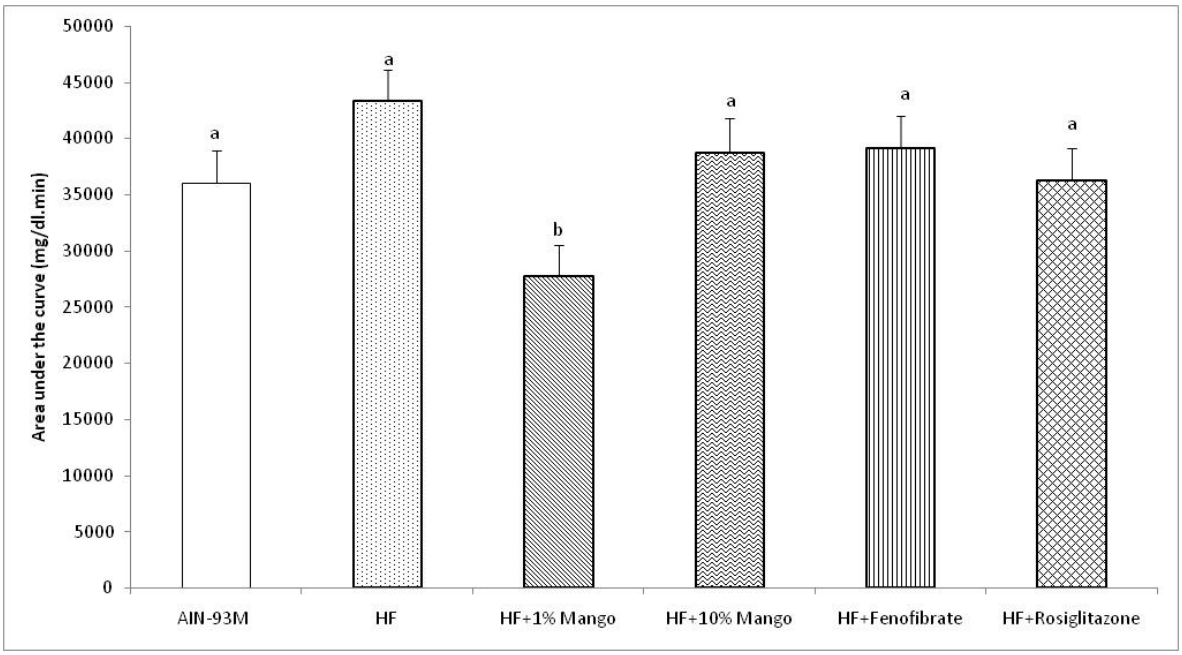
[1]Values are mean ± SE, n=6/group.

Figure 2: Effects of mango, rosiglitazone, and fenofibrate on % body fat of mice fed high fat (HF) diet for two months[1]



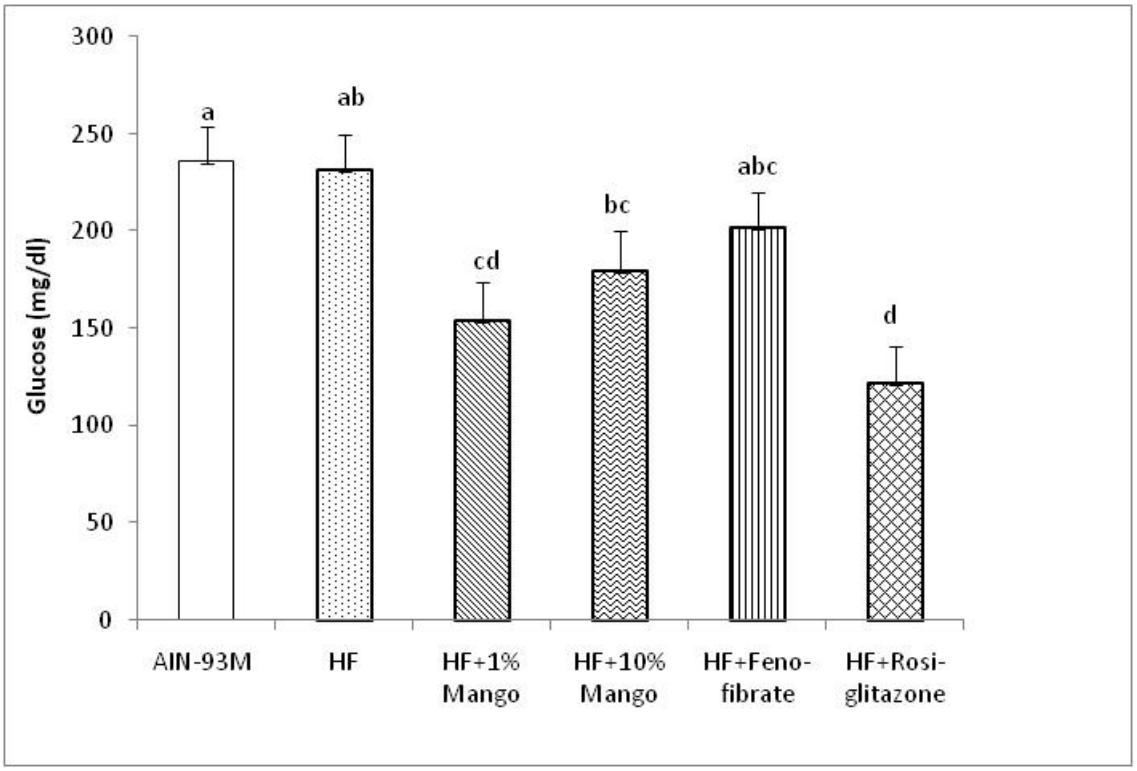
[1]Bars are mean ± SE, n=8/group; bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 3: Effects of mango, rosiglitazone, and fenofibrate on glucose area under the curve after a glucose tolerance test of mice fed high fat (HF) diet for two months[1]



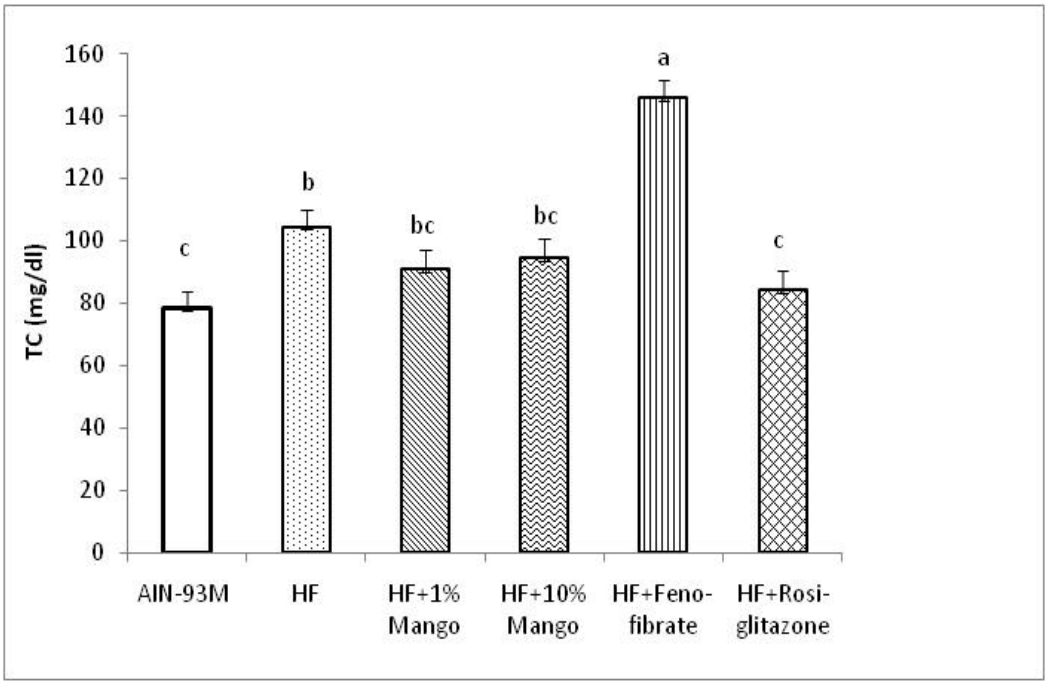
[1]Bars are mean ± SE, n=6/group; bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 4: Effects of mango, rosiglitazone and fenofibrate on plasma glucose level of mice fed high fat (HF) diet for two months[1]



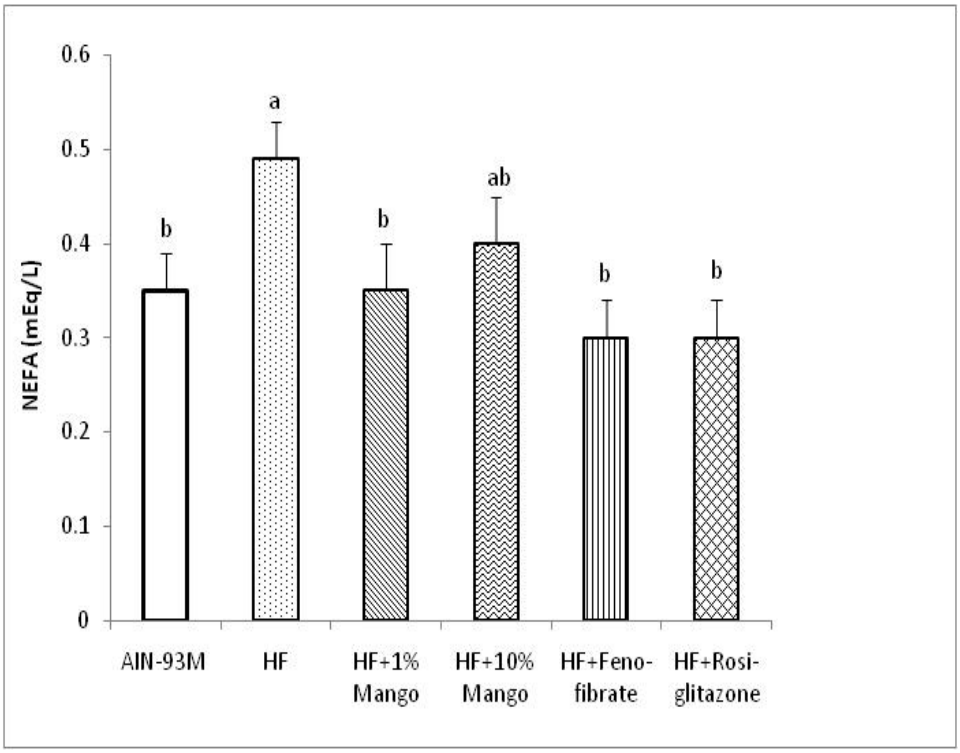
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 5: Effects of mango, rosiglitazone, and fenofibrate on plasma total cholesterol (TC) concentrations of mice fed high fat (HF) diet for two months[1]



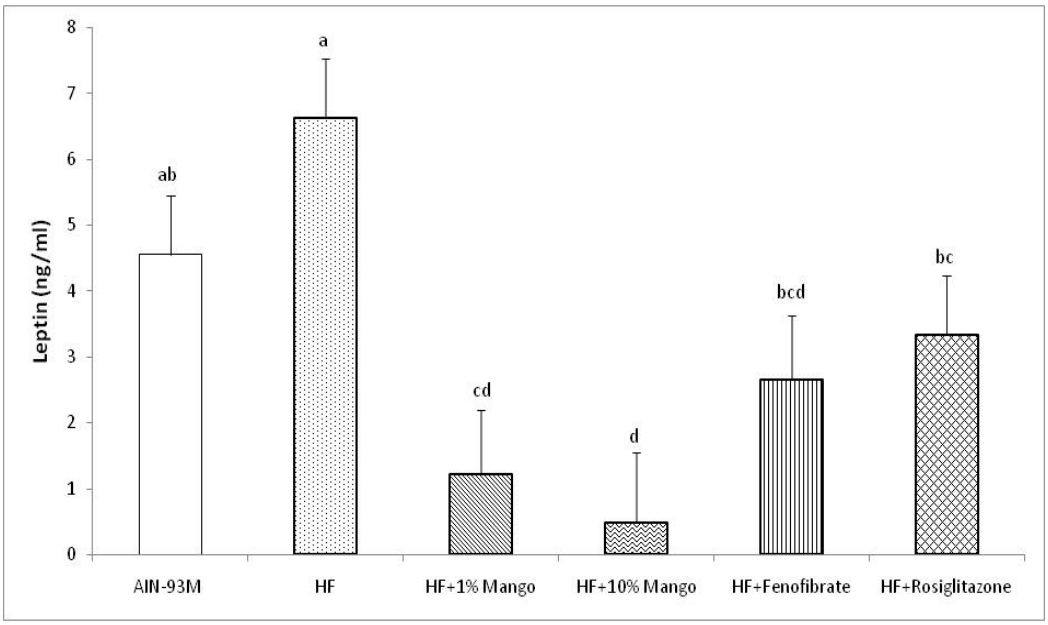
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 6: Effects of mango, rosiglitazone, and fenofibrate on plasma non-esterified fatty acid (NEFA) concentrations of mice fed high fat (HF) diet for two months[1]



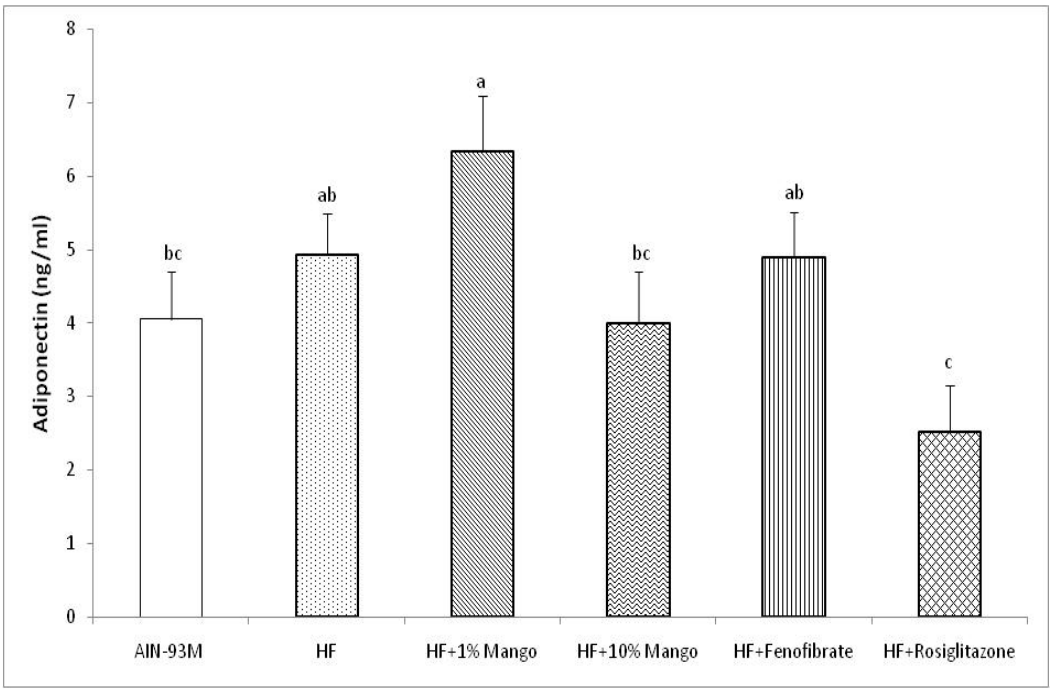
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 7: Effects of mango, rosiglitazone, and fenofibrate on plasma leptin concentrations of mice fed high fat (HF) diet for two months[1]



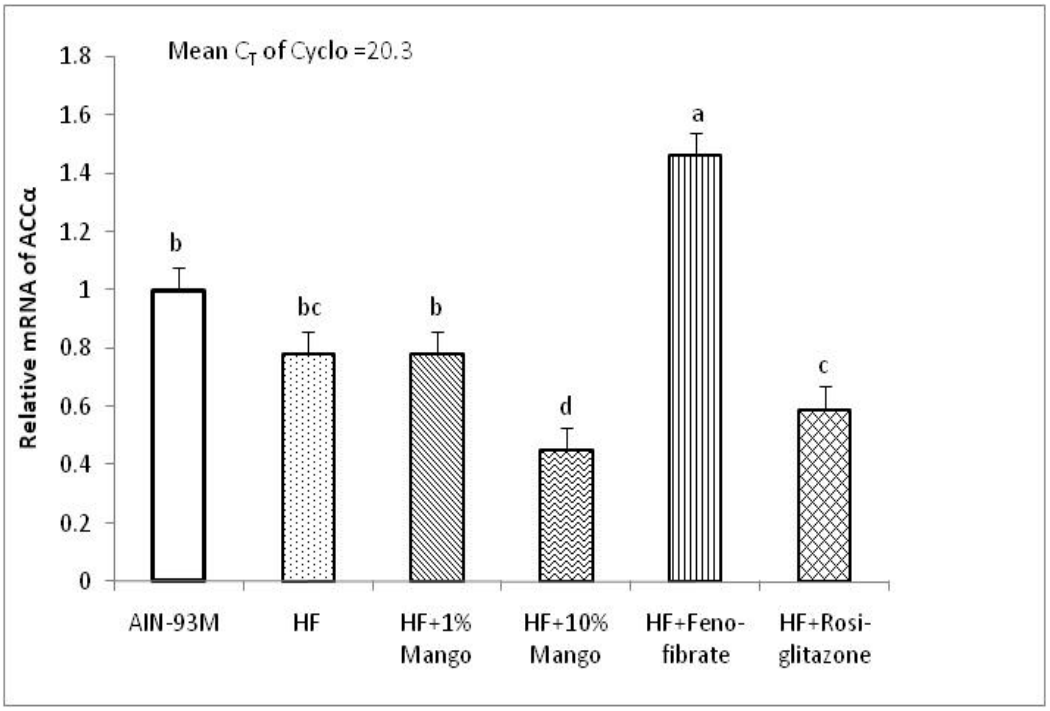
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 8: Effects of mango, rosiglitazone, and fenofibrate on plasma adiponectin concentrations of mice fed high fat (HF) diet for two months[1]



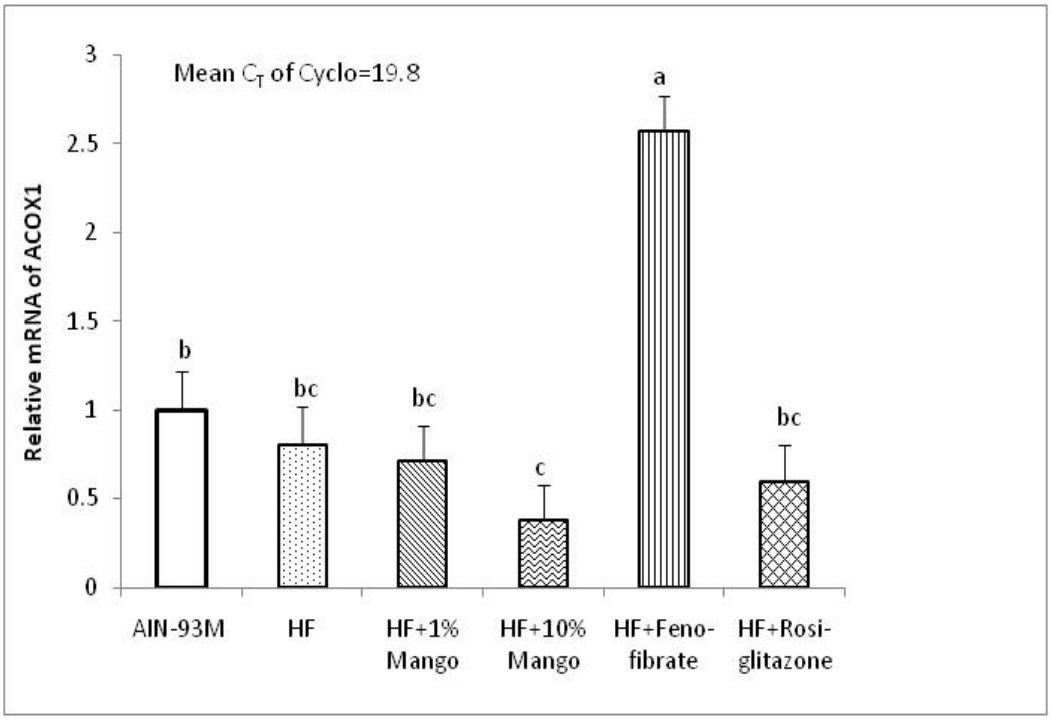
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 9: Effects of mango, rosiglitazone, and fenofibrate on relative mRNA of acetyl-CoA carboxylase alpha (ACCα) in the liver of mice fed high fat diet (HF) for two months[1]



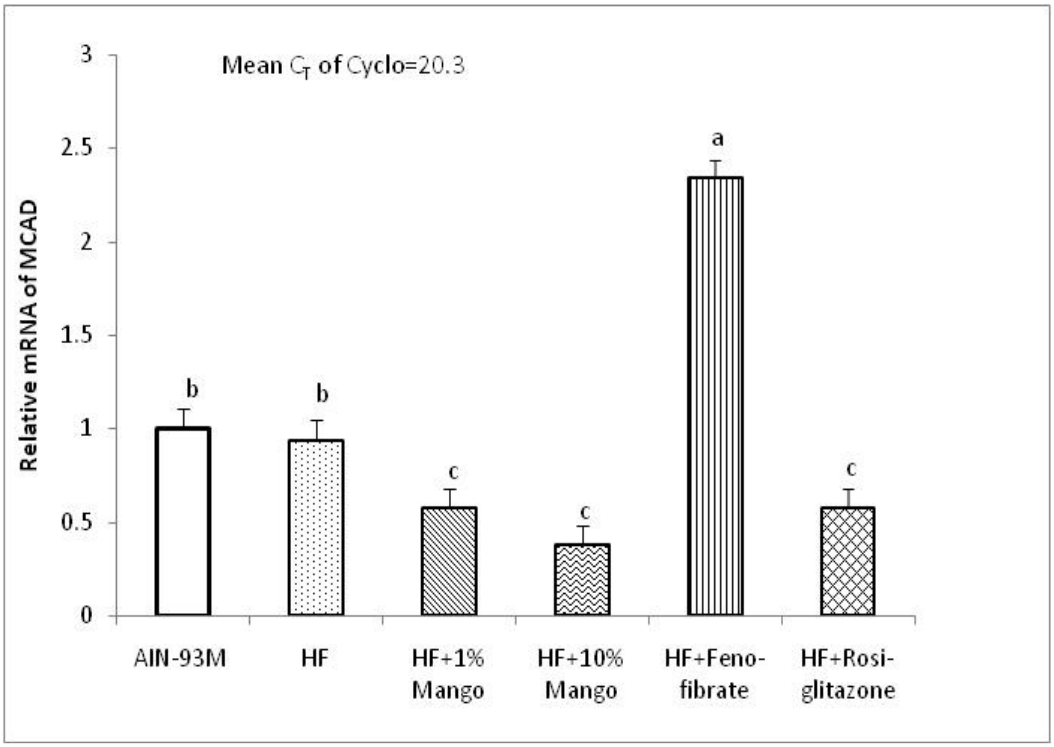
[1]Bars are mean ± SE, n=6/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 10: Effects of mango, rosiglitazone, and fenofibrate on relative mRNA of acyl-coenzyme A oxidase 1 (ACOX1) in the liver of mice fed high fat (HF) diet for two months[1]



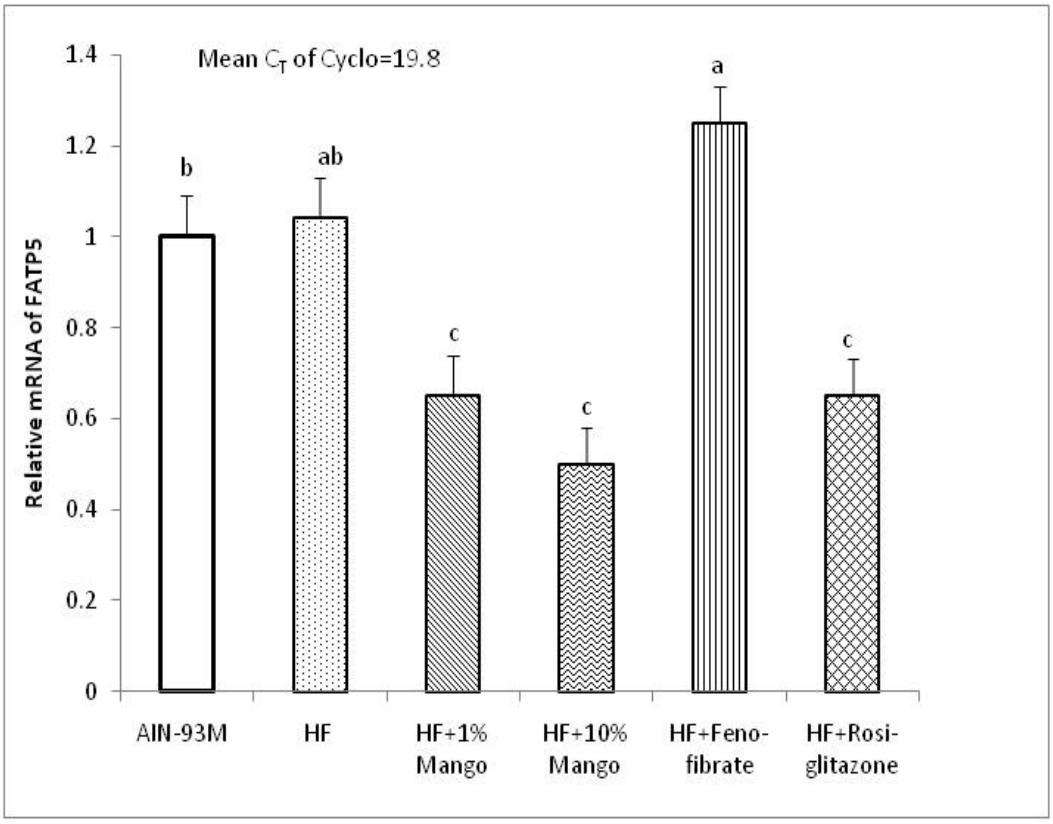
[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 11: Effects of mango, rosiglitazone, and fenofibrate on relative mRNA of medium-chain acyl dehydrogenase (MCAD) in the liver of mice fed high fat (HF) diet for two months[1]



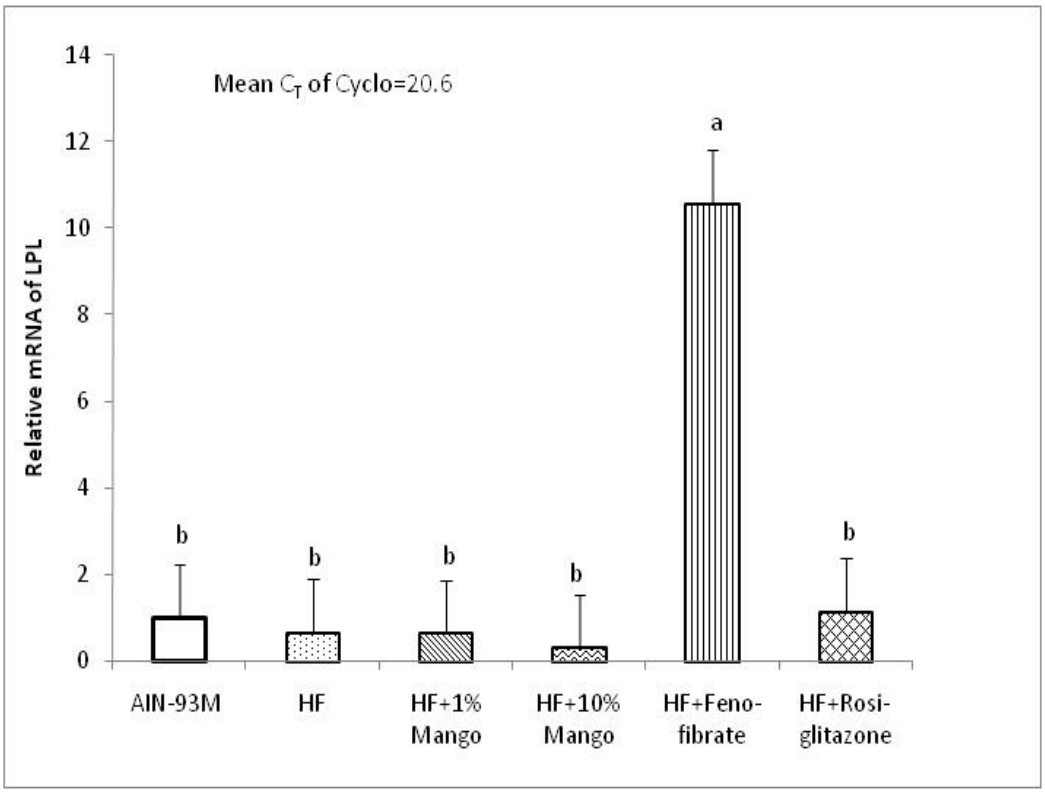
[1]Bars are mean ± SE, n=6/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 12: Effects of mango, rosiglitazone, and fenofibrate on relative mRNA of fatty acid transporter protein 5 (FATP5) in the liver of mice fed high fat diet (HF) for two months[1]



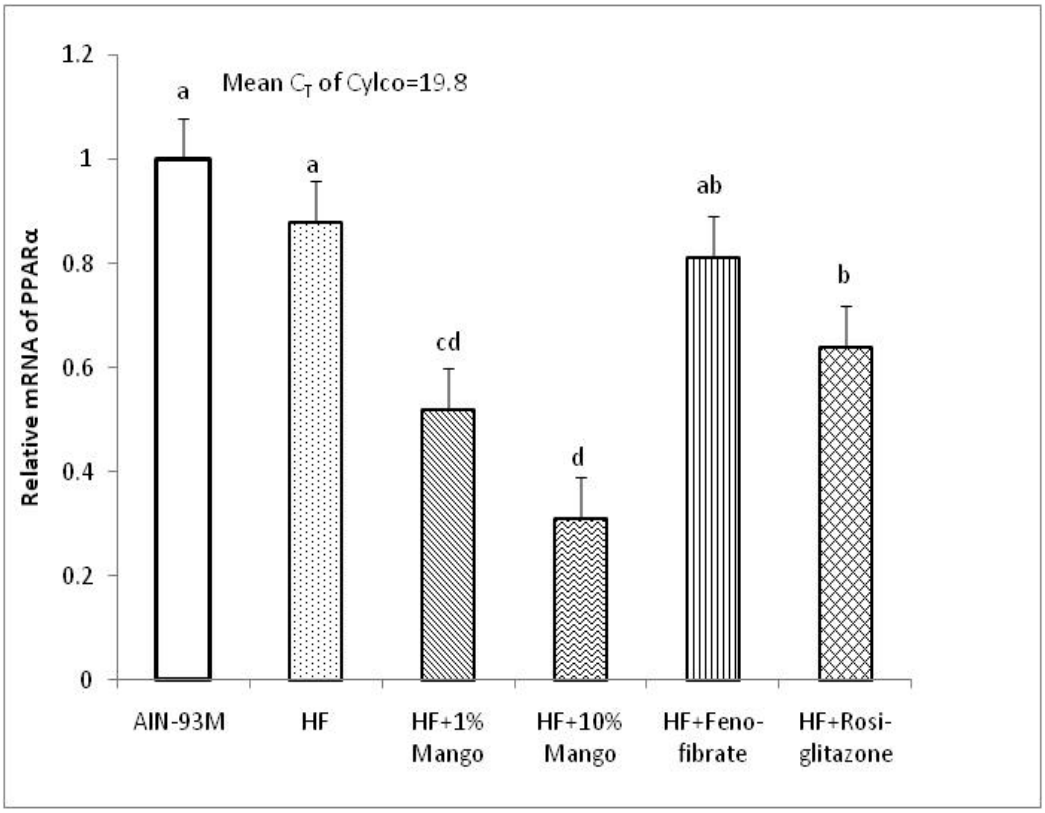
[1]Bars are mean ± SE, n=6/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 13: Effects of mango, rosiglitazone, and fenofibrate on relative mRNA of lipoprotein lipase (LPL) in the liver of mice fed high fat (HF) diet for two months[1]



[1]Bars are mean ± SE, n=6/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

Figure 14: Effects of mango, rosiglitazone and fenofibrate on relative mRNA level of peroxisome proliferator-activated receptor alpha (PPARα) in the liver of mice fed high fat (HF) diet for two months[1]



[1]Bars are mean ± SE, n=8/group; Bars that do not share the same letters are significantly ($P<0.05$) different from each other.

CHAPTER V

CONCLUSION

This study investigated the effect of two doses of freeze-dried mango on body composition and blood glucose and lipids in a mouse model of high fat diet-induced obesity. Moreover, the effects of mango were compared to that of fenofibrate and rosiglitazone, known PPAR agonists. This animal study lasted for about eight weeks and the experiment duration is slightly shorter than other studies fed animals with high fat diet [238-239]; however the effects of treatments had shown already in a short period.

In this study, we did not observe differences in weekly and final body weights among the treatment groups. This is likely due to the mice in the normal diet having significantly higher food intake than those in the high fat diet since mice were fed *ad libitum*. Moreover, all groups fed the high fat diet groups had similar food intake. Despite similar final body weights among the groups, there were differences in body composition, specifically fat mass and percent body fat. No difference in lean mass was observed. Fat mass and percent body fat was highest in the high fat group and the two doses of mango had comparable effect to that of rosiglitazone and fenofibrate in modulating body fat, which illustrated the benefit of mango supplements in reducing body fat. To our knowledge, this is the first study demonstrating the effectiveness of mango in reducing body fat.

The findings of our study that fenofibrate prevents adiposity is consistent with other animal studies [32, 240-241]. This effect of fenofibrate on adiposity is attributed to its role in regulating the synthesis and clearance of lipoprotein [161] and energy dissipation by induction of uncoupling protein in the liver mitochondria [242]. In our study, treatment with rosiglitazone reduced the weight of adipose tissue to the control group. This finding is consistent with the pleiotropic effects of TZDs modulating visceral fat while increasing subcutaneous fat [243]. Rosiglitazone is a known PPAR-γ agonist and is responsible for cell differentiation in adipose tissue [244] which may partly explain the decrease in adipose tissue mass observed in this study. If cell differentiation is limited by rosiglitazton, the growth of adipose tissue might be restricted.

Our study showed that 1% and 10% mango supplementation, similar to rosiglitazone and fenofibrate, reduced adipose tissue mass to that of the mice fed normal rodent diet (AIN-93M). The mechanism by which mango reduces fat mass may partly be due to its effect on upregulating the gene expression of aP2. Adipocyte protein 2 (aP2) is a marker of terminal adipocyte differentiation and involved in free fatty acid transport and shunting within the cell [245-246]. PPAR-γ agonists such as rosiglitazone upregulate the expression of aP2, a PPAR-γ target gene [247]. Our findings show that the aP2 gene expression tended ($P=0.0598$) to be elevated with rosiglitazone treatment (three times higher than the high fat diet fed mice not receiving rosiglitazone), however, a relatively low dose of rosiglitazone as was used in our study may be sufficient to augment the induction of lean phenotype as seen by Kuda and colleagues [248]. However, a ten fold higher dose of rosiglitazone has been shown to promote obesity, as observed in most studies with mice [249-250]. The reduced aP2 expression in the high fat diet fed animals

may be an adaptive response of the adipose tissue to limit further expansion of fat storage. The effects of mango and rosiglitazone on adipose mass may be by modulating the expression of a critical nuclear transcription factorthat can trigger the entire process of adipocyte differentiation. However, this is speculative and needs to be further explored.

In addition to its role in adipocyte differentiation, aP2 is also linked to inflammation and metabolic syndrome [246]. Circulating aP2 is correlated with insulin resistance and dyslipidemia in human studies [251-252]. aP2 deficiency is believed to promote insulin resistance in high fat diet induced obesity mouse model [253].

Liver weight and changes in expression of genes in the liver regulated by fenofibrate demonstrated the effectiveness of the dose of fenofibrate used in this study. Mice receiving fenofibrate had the highest liver weight among all the treatment groups, which is consistent with the findings in rats and mice [254]. Fenofibrate induces peroxisome proliferation in the liver which contributed to the increase in liver weight. Peroxisomes are organelles that contain many enzymes involved in fatty acid degradation [255] and PPAR-α agonist activates genes encoding these enzymes during dimerization with RXR [42, 256]. Low dose mango did not cause an increase in liver weight as seen with fenofibrate, while the high dose mango group had an intermediate effect on liver weight.

Glucose tolerance tests demonstrated that freeze-dried mango at l% dose exhibited glucose lowering properties in our animal model. This result is more pronounced than the one obtained from the rosiglitazone group, a known glucose-lowering agent. As a comparison, the 10% dose mango is similar to the rosiglitazone

group, but not as impressive as the 1% dose in lowering blood glucose. In addition to the glucose tolerance test, plasma glucose obtained at the end of the study confirmed the glucose-lowering properties of mango. Similar to the findings of the glucose tolerance test, the low dose mango had the most effect in reducing plasma glucose. This unique glucose lowering property of mango may be attributed to the increasing stimulation of pancreatic cells to secrete insulin [206] or the suppression of intestinal absorption of glucose [257]. To our knowledge, only three studies have been conducted that investigated the glucose lowering properties of mango and these studies have been limited to the extract of stem bark [207] or leaves [258]. The aqueous extract of mango leaves prevented hyperglycemia in STZ-induced diabetic rats [258]. Our findings are the first to demonstrate the effectiveness of mango pulp in modulating hyperglycemia induced by high fat diet.

There were no significant differences in the mRNA expression of GLUT4. Translocation of GLUT4 to the cell membrane promotes glucose uptake resulting to lower blood glucose concentrations [179]. The mechanism of how mango exactly performs glucose lowering effects is not clear at this time. Mango may affect genes involved in hepatic glycogenesis and gluconeogenesis and not necessarily uptake and genes involved in these pathways should be examined in future studies.

The component of mango responsible for this positive effect on glucose is not known at this time. In addition to the phenolic content of mango, a component that may have contributed to this glucose-lowering property of mango is dietary fiber. The mango used in this study contains approximately 4% dietary fiber. Dietary fiber has been shown to lower blood glucose level in laboratory animals [259] and in humans [260]. For

example, dietary fiber fractions from the plant, *Trigonella foenum graecum*, lowered blood glucose in a rat model of type 2 diabetes by interfering with the digestion of sucrose [261]. Similarly, cereal fiber lowered glucose level in diabetics [262].

The antioxidant content of mango may also help in reducing oxidative stress and lowering complications of diabetes. Mango is a significant source of the antioxidants β-carotene and C [23]. Increased amount of fatty acids and modified lipoproteins can induce oxidative stress as a result of inflammatory responses, and the production of free radicals promote the complications associated with type 2 diabetes [263]. Several studies have reported that depletion of antioxidants (i.e; tocopherols, carotene, ascorbic acids, lycopene) is involved in cardiovascular and diabetes complications [264-267]. These antioxidants have inhibitory effect on ROS generation by suppressing the activity of ROS producing enzymes such as xanthine oxidase, cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxidase, NADH oxidase [268]. The effect of mango in reducing oxidative stress needs to be further explored.

In addition to lowering glucose, mango had moderate effects on plasma total cholesterol in this animal model. Mice receiving the two doses of mango or rosiglitazone had similar total cholesterol to those receiving the normal diet. However, plasma total cholesterol of the mango group was not statistically different from those receiving the high fat diet. Interestingly, fenofibrate produced an increase in plasma total cholesterol and has no effect on triglycerides, which is contradictory to the findings of some earlier studies [168, 170, 176-177]. Fenofibrate is a known hypolipidemic drug that has been used to manipulate lipid metabolism since 1980 [160]. It activates lipoprotein lipase to reduce the generation of triglycerides in the liver in mice [175-176].

The fenofibrate group also showed the highest plasma HDL-C concentration which is consistent with earlier findings [170]. Moreover, the fenofibrate group exhibited significantly higher liver gene expression of ACCα, ACOX1, FATP5, LPL and MCAD, again consistent with other studies [155, 241]. Changes in the gene expression in the liver explain the observed effect on lipids by fenofibrate. Whether mango acts similarly as fenofibrate in modulating lipids is not clear at this time.

Futher studies may need to focus on genes involved in glucose metabolism that could investigate the hypoglycemic effect of mango. Overall, low dose mango was better than the glucose lowering drug, rosiglitazone, in modulating blood glucose concentration due to high fat diet. Moreover, mango similar to rosiglitazone favorably alters body composition by reducing % body fat without affecting lean mass. Human studies need to be conducted to confirm our findings.

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APPENDIX

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 7/29/2010

Date : Monday, July 30, 2007 Animal Care and Use Protocol (ACUP) No : HE074

Proposal Title: Mango modulates glucose and lipid parameters in high fat diet-induced obesity

Principal Investigator:

Edralin Lucas
Nutritional Sciences
425 HES
Campus

Reviewed and Processed as: Full Committee

Approval Status Recommended by Reviewer(s) : Approved

The protocol as revised is approved. You are approved for a total of 150 mice for the next three years.

Signatures:

Charlotte Ownby, IACUC Chair

Monday, July 30, 2007
Date

cc: Department Head, Nutritional Sciences
Director, Animal Resources

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

VITA

WENJIA LI

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF MANGO ON GLUCOSE AND LIPID PARAMETERS OF MICE FED HIGH FAT DIET

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in TianJin, P.R.China, on May 29, 1983, the daughter of Li, Zheng and Guo, Jinying.

Education: Graduted from No. 43 High School, Tianjin, P.R. China in July 1998; received Bachelor of Science degree in Biological Sciences from University of Guelph, Guelph, Ontario, Canada in June 2006. Completed the requirements for the Master of Science in your major at Oklahoma State University, Stillwater, Oklahoma in December, 2009.

Experience: Employed by Oklahoma State University, Department of Nutritional Sciences as a graduate research assistant; Oklahoma State Department of Nutritional Sciences, 2007-2009.

Name: WENJIA LI Date of Degree: December, 2009

Institution: Oklahoma State University Location: OKC or Stillwater, Oklahoma

Title of Study: THE EFFECTS OF MANGO ON GLUCOSE AND LIPID

PARAMETERS OF MICE FED HIGH FAT DIET

Pages in Study: 99 Candidate for the Degree of Master of Science

Major Field: Functional foods and chronic diseases

Scope and Method of Study: Mango is a tropical fruit that contains bioactive compounds that may reduce cardiovascular disease (CVD) risk factors. To our knowledge, limited studies have been conducted to investigate the effect of mango flesh on body composition and glucose and lipid parameters. Therefore, this study compared the effect of mango with known agonist of peroxisome proliferator activated-receptor (PPAR), fenofibrate (PPAR-α agonist) and rosiglitazone (PPAR-γ agonist), in modulating body fat, and plasma glucose and lipid parameters in mice fed high fat diet. Male C57BL6 mice were randomly assigned into one of six treatment groups (n=8/group) for two months: high fat diet (HF), HF + fenofibrate (500 mg/kg diet), HF + rosiglitazone (50 mg/kg), HF + 1% mango, and HF + 10% mango. High fat diet significantly increased adipose tissue weight and 1% mango significantly reduced the weights of adipose tissue similar to normal diet, 10% mango, and fenofibrate and rosiglitazone groups. Mice receiving 1% mango groups had the lowest area under the curve after a glucose tolerance test. The two doses of mango significantly reduced plasma non-esterified fatty acids to the level of normal diet group.

Findings and Conclusions: Supplementation with 1% mango for 8 weeks was the most effective in modulating glucose and lipid levels due to high fat diet. Our findings suggest that incorporation of mango in the diet reduces CVD risk factors in this animal model and warrants further investigation in humans.

ADVISER'S APPROVAL: Dr. Edralin A. Lucas