THE EFFECTS OF MANGO SUPPLEMENTATION ON CLINICAL PARAMETERS OF INDIVIDUALS WITH MODERATELY ELEVATED BLOOD GLUCOSE

By

JESSICA SEMKOFF

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Oklahoma State University

Stillwater, OK

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Thesis Approved:

Dr. Edralin Lucas

Thesis Adviser

Dr. Brenda Smith

Dr. Stephen Clarke

Name: JESSICA SEMKOFF

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Abstract:

Diabetes is the seventh leading cause of death in the U.S. and is currently estimated to affect 25.8 million people. Type 2 diabetes or adult-onset diabetes is prevalent due to the rise in obesity. Pre-diabetes (fasting blood glucose between 100-125 mg/dl) is a condition with an elevated blood glucose that is not high enough to be categorized as diabetes. Dietary intervention in individuals with moderately elevated blood glucose may help delay the development of diabetes. The consumption of fruits and vegetables has been investigated for their effect on chronic diseases like diabetes. This study investigated the effects of supplementation of freeze-dried mango on clinical parameters of individuals with moderately elevated blood glucose. The mango fruit is rich in vitamins and minerals as well as the phenolic compounds quercetin and mangiferin that possess anti-inflammatory and antioxidant properties. In a crossover design, individuals with fasting blood glucose between 100-125 mg/dl were recruited and asked to consume either a freeze-dried mango (10 g) or a placebo daily for 12 weeks with 3-4 weeks wash-out period between treatments. After 12 weeks of supplementation, there were no significant differences in glycemic indices between mango and placebo groups. There were also no significant differences in body composition or clinical parameters in either group with time or treatment. Unlike our earlier findings, chronic consumption of the mango fruit has a modest effect in lowering blood glucose but does not cause hyperglycemia in individuals with moderately elevated blood glucose.

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CHAPTER I

INTRODUCTION

The Center for Disease Control (CDC) reported that in the last 20 years there has been an increase in obesity in the United States, affecting all regions in the country and individuals of all ages [1]. They reported that over one-third of U.S. adults and 17% of children and adolescents are obese [1]. The CDC defines obesity as a range of weight that is greater than what is considered healthy for a given height or a body mass index (BMI) > 30 kg/m^2 [1]. As obesity increases, other health-issues such as type 2 diabetes (T2D) also increase. Diabetes is preceded by pre-diabetes, a condition where blood glucose levels are higher than normal (i.e., fasting blood glucose 100 to 125mg/dL), but not high enough to be considered for diabetes (fasting blood glucose ≥ 125 mg/dL) [2]. Of those diagnosed as pre-diabetic, 15% to 30% will develop T2D within the next five years if lifestyle changes are not implemented to improve their condition [1].

To explain the pathophysiology of diabetes, a basic understanding of carbohydrate metabolism and the role that insulin plays is important to discuss. Under normal conditions, postprandial glucose signals the release of insulin from the beta cells of the pancreas to normalize blood glucose. The hormone, insulin, binds to a cell membrane receptor and induces a

signaling cascade that leads to the translocation of the glucose transporter to the cell membrane of insulin-sensitive tissues such as skeletal muscle and adipose tissue. This glucose transporter facilitates the entry of glucose into the cell, which then leads to normalization of blood glucose levels. Three actions happen in T2D: peripheral resistance to insulin, altered production of insulin in pancreatic beta cells, or an increased production of hepatic glucose. These alterations can stimulate the progression of each other leading to the development of T2D [3]. Alterations in insulin production contributing to the disease can be seen with changes in incretin hormones such as gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1). Along with insulin's primary effects on glucose homeostasis, it also plays a role in many other cellular events such as lipid metabolism, glycogen synthesis, gene transcription, protein synthesis and degradation and DNA synthesis [4].

Obesity, sedentary lifestyle and dietary habits play a role in the development of T2D. Weight loss is one of the important lifestyle modifications for those with elevated blood glucose. Excess adipose tissue can hinder glucose uptake and contribute to insulin resistance that results to high blood glucose levels. This occurs in part because adipose tissue secretes inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) which impairs insulin signaling. Exercise has been shown to promote glucose uptake, alleviating the hyperglycemic burden on many diabetic patients. Richter and colleagues assessed plasma insulin levels after exercise sessions and found that a single exercise session can increase skeletal muscle's response to insulin [5]. Therefore, both exercise and weight loss are important in the prevention and management of T2D. In addition to exercise, calorie restriction can contribute to weight loss. It is recommended that a moderate calorie restriction of 250-500 calories per day can delay or prevent the development of T2D [6].

In addition to lifestyle changes, pharmacological options are also available for managing blood glucose. Commonly used diabetes medications act through three general mechanisms of

action; 1) increase insulin secretion like sulfonylureas and meglitinides, 2) improve insulin action like metformin and thiazoledinediones (TZDs), and 3) reduce glucose absorption by preventing the break-down of carbohydrates like alpha glucosidase inhibitors. Metformin is the most commonly used medication for T2D and acts by reducing fasting hepatic glucose output and improving peripheral insulin resistance [7]. Pharmacological options for diabetes are effective in modulating blood glucose; however, it is associated with unwanted side effects ranging from gastrointestinal effects and weight gain to renal and cardiovascular risks [7]. Therefore, alternative approaches for the management of T2D are needed.

A diet containing a variety of fruits and vegetables has been reported to reduce the risk of many chronic diseases including T2D [8]. Fruits and vegetables contain bioactive compounds that can help delay the development of many chronic conditions. For example, a clinical study demonstrated that supplementation of whole blueberry powder for six weeks improved insulin sensitivity in obese, non-diabetic, and insulin-resistant individuals [9]. One variety of lettuce, Rutgers Scarlet lettuce, has anti-diabetic properties because of its ability to stabilize glucose levels and increase insulin sensitivity [10]. A meta-analysis found that greater consumption of blueberries, grapes, apples, bananas, and grapefruit are associated with reduced risk of T2D [8]. The positive effects of these fruit and vegetables on improving aspects of T2D are mostly attributed to the different polyphenols and antioxidants.

One fruit that is rich in bioactive components such as vitamin C, beta-carotene, phosphorus, and potassium, as well as fiber and phenolic compounds that have been shown to have health benefits is the mango fruit. The phenolic compounds quercetin and mangiferin found in mango have been shown to positively affect blood glucose concentrations [11]. Quercetin was shown to increase the number of pancreatic islet cells, increase insulin release and reduce plasma glucose in streptozocin (STZ)-induced diabetic rats [12]. Duang and colleagues speculated that the glucose-lowering effect of mangiferin is by hindering glucose absorption from the intestine [13].

Muruganandan and colleagues also suggested that mangiferin decreases glucose absorption from the intestine [14]. This assumption is supported by the findings of Yoshikawa and colleagues that reported mangiferin inhibits α-glucosidase enzymes involved in the digestion of carbohydrates [14, 15]. The bioactive compounds in mango are shown to decrease oxidative stress [13, 16, 17]. Increased oxidative stress through the production of reactive oxygen species (ROS) is often observed in diabetes [18]. These reactive species can alter the structure or function of cellular proteins, lipids, and nucleic acids and can also trigger the activation of stress signaling pathways like the serine/threonine kinase cascade which can lead to insulin resistance [18].

Most of the studies on the health effects of mango have been on the phenolic compounds (mangiferin and quercetin) extracted from the different parts of the mango plant or flesh of the mango fruit. Limited studies have investigated the health effects of the pulp of the mango fruit. A study by Lucas and colleagues investigated the effects of freeze-dried mango pulp on adiposity, glucose metabolism, and lipid profiles in mice fed a high fat diet [19]. The effects of mango were also compared to the hypolipodemic drug (i.e. fenofibrate) and hypoglycemic drug (i.e. rosiglitazone). The investigators reported that mango was comparable to fenofibrate and rosiglitazone in preventing increases in fat mass and percent body fat and also decreased insulin resistance, improving insulin sensitivity [19]. Guevarra *et al*. compared the glycemic response of mango to that of other tropical fruits (chico, pineapple, and papaya) in T2D individuals [11]. Chico and mango induced a lower blood glucose response compared to pineapple and papaya, and this was attributed to the fiber and other nutrients found in these fruits contributing to the delay in their rate of digestion and absorption [11]. These findings give validity to the idea that mango is an acceptable fruit for diabetic patients to consume because it does not cause a rapid increase in blood sugar. More research needs to be done to investigate the effects of whole mango fruit on clinical parameters of individuals with elevated blood glucose. Moreover, research investigating

the appropriate dose of mango supplementation administered to individuals with elevated blood glucose is needed.

This study investigated the effects of chronic mango consumption in modulating blood glucose levels in individuals with elevated blood glucose. Based on previous animal and limited human studies, we ***hypothesize*** that freeze-dried mango and its bioactive components will improve blood glucose concentrations in individuals with moderately elevated blood glucose. The ***specific aims*** of the study are to investigate the effects of daily supplementation of freeze-dried mango (10g/d) for 3 months on

1. parameters of glucose homeostasis
2. anthropometrics, and
3. inflammatory markers and antioxidant status of individuals with moderately elevated blood glucose.

The aims were achieved using a cross-over study design of twenty five individuals with moderately elevated blood glucose. They were asked to consume 10 g/d of freeze-dried mango or placebo for 3 months with a 3-4 weeks washout period in between treatments. Blood glucose, body composition, inflammatory markers and antioxidant status were assessed at baseline and at the end of treatment period.

Some of the *limitations* of our study include: (1) we cannot be certain that the supplement was consumed daily; (2) the dose of mango (10g/d) was a relatively small amount compared to some previous studies [14, 60, 68]; (3) a 3 month intervention period might not be enough time for the supplementation to cause significant changes; and (4) a home testing glucometer was used for screening the participants instead of glycosylated hemoglobin (HgbA1C) values.

CHAPTER II

REVIEW OF LITERATURE

Prevalence of obesity, pre-diabetes, and diabetes

Obesity is a health concern in the US and worldwide as its prevalence increases every year. In the United States, there was a dramatic increase in obesity in the twenty year span between 1990 and 2010. In 2010, there were twelve states that recorded their obesity prevalence to be greater than 30% compared to no states having an obesity prevalence of 30% or more ten years earlier [1]. More than 35.7% of US adults and approximately 17% of children and adolescents were obese in 2010. Health conditions related to obesity include heart disease, stroke, certain cancers, and type II diabetes (T2D). It is estimated that the medical cost of obesity in the US in 2008 was $147 billion. As the incidence of obesity grows so do the medical costs associated with it [1]. It is estimated that medical costs for obese people were $1,429 higher per year compared to those of normal weight.

The obesity epidemic has resulted in an increase in the prevalence of pre-diabetes and T2D. Weight gain has been found to be one of the most important risk factors in the development of T2D [20]. In 2012, there were a total of 29.1 million people in the US with diabetes [1]. The estimated total cost of diabetes in the US as of 2012 was $245 billion [1]. Therefore, intervention strategies are needed to reduce the incidence and cost associated with T2D.

Obesity and diabetes

Adipose tissue is a normal part of the human body and acts as an energy reservoir. The body stores any excess energy in adipose tissue to be used during periods of lower energy intake. Adipocytes can expand by increasing either in size (hypertrophy) or number (hyperplasia) of fat cells. Other functions of the adipose tissue include: 1) protecting organs from mechanical damage, 2) secreting a variety of bioactive peptides and adipokines, which can act by autocrine, paracrine, and endocrine mechanism, and 3) their involvement in many biological processes including neuroendocrine and immune function [21, 22]. Although adipose tissue performs important functions in the body, excess amounts can have detrimental health effects. Excess adipose tissue or obesity is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, and pro-thrombotic pro-inflammatory states [22].

Adipose tissue secretes adipokines that have endocrine functions. Leptin, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), resistin, visfatin and adiponectin are proteins secreted from adipose tissue that add to the complex relationship of obesity, insulin resistance, and inflammation. Leptin plays a role in regulating appetite and metabolism [23]. The amount of leptin secreted is proportional to the adipose tissue mass. Under normal conditions, leptin regulates body weight and food intake, but in an obese individual, there seems to be resistance or insensitivity to leptin [24].

Contradicting its role in cachexia, TNF-α was the first adipose derived factor shown to link obesity, inflammation, and diabetes [22, 24]. TNF-α, manly produced by macrophages, has been found to increase in obesity and is positively related to insulin resistance [22]. It is reported that in adipose tissue, TNF-α indirectly represses the genes associated with the uptake and storage of non-esterified fatty acids and glucose [22]. The mechanism by which TNF-α contributes to insulin resistance is by hindering the uptake of glucose in peripheral tissues. Kinase activity involved in

the initial steps in insulin signaling is inhibited by TNF-α [25]. TNF-α not only acts in adipose tissue, but also in the liver by reducing glucose uptake and metabolism and impairs insulin signaling [22].

IL-6 is another cytokine secreted from the adipose tissue and is positively correlated to obesity, impaired glucose tolerance, and insulin resistance [22]. As much as, one third of circulating IL-6 comes from the adipose tissue [22]. IL-6 decreases insulin signaling by down-regulating expression of insulin receptor signaling components and suppressor of cytokine production [22]. Therefore, elevated IL-6 concentration is implicated in obesity and insulin resistance and is a predictor for the development of T2D as well as CVD [22].

Adiponectin, unlike leptin, TNF-α, and IL-6, has a strong inverse association with insulin resistance and the inflammatory state. Serum adiponectin levels are low when there is insulin resistance from obesity or lipodystrophy, and administration of adiponectin improves metabolic parameters in obesity or lipodystrophy [22, 26]. Adiponectin concentrations are higher with weight reduction and improved insulin sensitivity [22]. In the liver, adiponectin increases insulin sensitivity by regulating fatty acid metabolism through increasing fatty acid oxidation, and decreasing circulating non-esterified fatty acids [22]. Adiponectin also suppresses hepatic glucose output aiding insulin sensitivity [22]. In the muscle, adiponectin stimulates glucose and fatty acid oxidation suggesting anti-diabetic effects of adiponectin [22].

In addition to adipokines, adipocytes also play a role in insulin resistance and development of T2D by secreting non-esterified fatty acids and glycerol. Chronically elevated circulating free fatty acids and glycerol induce insulin resistance in skeletal muscle by inhibiting glucose transport activity [27]. This results in increased lipid oxidation and decreased glucose uptake contributing to insulin resistance [27]. The inhibition of glycogen synthase activity may be a contributor in the decrease mobilization of glycogen [27].

Pre-diabetes and diabetes

Blood glucose levels are tightly regulated in healthy individuals, but as an individual advances toward T2D, defects in glucose homeostasis develop and over many years these small changes can lead to the development of T2D. This metabolic condition is characterized by disordered fuel homeostasis with hyperglycemia and altered lipid metabolism due to pancreatic islet β-cells not being able to secrete sufficient insulin to appropriately respond to the body's needs during over-nutrition, inactivity, obesity, and insulin resistance [28]. Under normal conditions when blood glucose levels are maintained, tissues like the liver, skeletal muscle, and heart are given optimal conditions to function properly. However, when blood glucose levels are not controlled and there is a chronic surplus, tissue and cell such as the pancreatic islet cells become overworked and over time become damaged. In addition to the pancreas, other tissues are also affected such as the adipose tissue, liver, skeletal muscle, and heart [28].

Pre-diabetics are individuals that have impaired fasting blood glucose (100 to 125 mg/dL) or impaired glucose tolerance and are at an increased risk of T2D [20]. Pre-diabetes is the early stage of T2D and experiences impaired glucose tolerance but no symptoms are observed. Impaired glucose tolerance is characterized by increased insulin resistance in skeletal muscle and liver as well as altered β-cell function [20]. As glucose concentration increases, the demand for insulin exhausts the β-cell resulting in impaired function. The combination of insulin resistance and impaired β-cell function is responsible for the increased risk of T2D in pre-diabetics [20].

In addition to impaired β-cell function, increased hepatic gluconeogenesis and glycogenolysis add to the surplus of glucose in circulation, which aggravates hyperglycemia. Impaired insulin secretion further allows for more glucose in circulation because insulin is not present to assist entry of glucose into tissues. When the body becomes resistant to insulin, tissues are incapable of using insulin to effectively transport and metabolize glucose, which causes the

pancreas to compensate by increasing production of insulin which can then lead to hyperinsulinemia and further contribute to the development of T2D [29].

The earliest sign of T2D is elevation of postprandial glucose, while also experiencing progressive insulin resistance. Under normal conditions, the body compensates with islet cell hypertrophy in order to increase insulin production to normalize circulating glucose, but in the progression of T2D, insulin production is insufficient to maintain euglycemia. This disrupted process further contributes to a high glucose load after a meal, which adds to the stress put on the beta cell to produce more insulin and contributes to the progression of this disorder [29]. An overload of fuel in the form of glucose is what drives the development of T2D [28].

Postprandial glucose is regulated by insulin secretion and suppression of hepatic gluconeogenesis and glycogenolysis. Insulin promotes glucose uptake into muscle and peripheral tissues to be used for energy or stored as glycogen. In T2D, glucose uptake by tissues is impaired and hepatic gluconeogenesis is not suppressed resulting in hyperglycemia. Under normal conditions, postprandial glucose stimulates insulin release from the beta cell. Insulin then binds to the cell surface receptor to allow glucose entry into the cell. Individuals with T2D have slightly diminished insulin receptor binding affinity and the cells do not respond to the presence of insulin and therefore do not allow the entry of glucose into the cell [29]. The glucose transporter, GLUT4 is sensitive to insulin signaling and allows glucose entry into the cell. GLUT4 is found in high concentrations in skeletal muscle and adipose cells [29]. In T2D, even though there is normal level of GLUT4, there is impaired glucose transport lending to the idea that there is abnormal insulin receptor binding or the translocation of GLUT4 to the cell surface is flawed [29]. The defect in the signaling pathway between insulin receptor and GLUT4 translocation results in insulin resistance [29]. With down regulation of glucose transport system using GLUT4, there is an increase in plasma glucose concentration. Increases of plasma glucose concentration of an extra 50-100 mg/dL

spanning for as little as 24 hours decreases GLUT4 translocation, significantly increasing insulin resistance [29].

Insulin resistance is commonly the earliest indicator in the development of T2D [29]. A small amount of insulin resistance is normal for the body to use during times of short-term overfeeding. During these times, skeletal and cardiac muscles develop insulin resistance to allow storage of excess nutrients in adipose tissue rather than having excess nutrients accumulate in other tissues contributing to steatosis and other metabolic stress [28]. T2D is characterized by insulin resistance that results in hyperglycemia. Hyperglycemia contributes to beta cell deterioration and further exacerbates the disorder [29]. As beta cells deteriorate, less insulin is able to be made and subsequently more hyperglycemia is expressed contributing to the vicious cycle.

Not only is postprandial glucose elevated in T2D, but individuals also experience fasting hyperglycemia. This is due to an increase in hepatic glucose output via increases in glycogenolysis and gluconeogenesis, because insulin is not being used effectively and is unable to signal for the suppression of hepatic glucose output [29]. The progression of the disease and lack of glycemic control is exacerbated by the ongoing deterioration of beta cell function adding to the decrease in insulin production [29]. The glucose toxicity theory suggests that continual exposure of the pancreas to small increases in blood glucose over a long amount of time can have adverse effects on β-cells [30].

Glucose toxicity of the pancreatic islet is defined as non-physiological and potentially irreversible β-cell damage caused by chronic exposure to supra-physiological glucose concentrations [30]. High concentrations of glucose in the blood result in desensitization and exhaustion of the pancreatic β-cell due to chronic glucose stimulation [30]. Another effect of high glucose concentration on the pancreas is β-cell exhaustion [30]. Glucose toxicity is associated with

chronic oxidative stress which damages cellular components of insulin production resulting in decreased insulin secretion [30].

Oxidative stress and diabetes

Oxidative stress due to increase production of reactive oxygen species (ROS) is also implicated in T2D [30]. ROS are involved in many redox-regulatory mechanisms of cells to maintain a consistent redox-homeostasis, but can drastically increase due to certain factors such as mitochondrial stress, environment and diet. To balance the production and removal of ROS, antioxidant defenses are needed to scavenge the ROS [31]. In a reduced antioxidant status, ROS react with phospholipids of membranes causing membrane permeability and instability as well as inhibiting cell signaling contributing to problems such as insulin resistance [31]. ROS can inactivate or degrade signaling proteins causing a disruption in insulin signal transduction resulting in insulin resistance [35]. ROS may also activate nuclear factors leading to the production of other pro-inflammatory cytokines, enhancing inflammation and generating other ROS. Obesity, a risk factor for T2D, is characterized by increased inflammation and cytokine production which aggravates oxidative stress [31].

Hyperglycemia also plays a role in oxidative stress. High concentrations of glucose increase the mitochondrial proton gradient, which results in an over-production of electron donors by pathways like the tricarboxylic acid cycle (TCA) and electron transport chain [32]. Chronic high glucose concentration increases production of mitochondrial superoxides that can damage tissues. One target of free radical reactions are unsaturated bonds in lipid membranes resulting in the loss of membrane fluidity and alignment. This damage culminates in inactivation, cross-linking, and denaturation of proteins [33]. Majority of glucose uptake occurs in the skeletal muscle which activates the mitochondrial electron transport chain for energy production that can also lead to production of superoxides. Not all of the ROS can diffuse across the mitochondrial membrane

and therefore accumulate in the mitochondria causing degradation and damage to the organelle [35]. Free radicals that are not neutralized by antioxidants can cause damage to DNA, lipids, proteins, and vital cellular structures [29]. Pancreatic islet cells are especially vulnerable to ROS because they have a low level of antioxidants enzyme to neutralize the harmful ROS [32].

Management of T2D

Lifestyle modification is recommended to decrease the risk of developing T2D. Weight loss through diet and exercise are encouraged as prevention methods because two of the main determinants in developing insulin resistance are obesity and lack of physical activity [36].

Weight loss due to calorie restriction increases oxidative capacity of skeletal muscle, thus preventing lipid accumulation in the skeletal muscle and improving insulin sensitivity. Calorie restriction reduces free fatty acids (FFA), which is beneficial because an excess of FFA promotes the accumulation of long-chain fatty acyl CoA, diacyglycerol and triglycerides that may promote the activity of protein kinase C and lead to insulin resistance [36]. Therefore calorie restriction induced weight loss has positive effects by decreasing free fatty acid levels, which also contribute to improvements in insulin sensitivity [36].

Exercise is another lifestyle factor that promotes insulin sensitivity. Exercise does not always result in weight loss but physical activity is still accompanied by improved insulin sensitivity in the absence of weight loss [36]. The changes, though not accompanied by a reduction in body weight or a decrease in fat composition, are likely due to increased GLUT 4 content [36]. Exercise that results in weight loss has a greater effect on insulin-stimulated glucose disposal [36]. Improvements in insulin-stimulated glucose disposal due to exercise are usually short lived and diminish within a week of exercise cessation [36].

Calorie reduction and exercise are lifestyle modifications for preventing T2D, but once T2D has developed, more aggressive treatment options such as pharmacologic options might be

necessary. There are three ways these pharmacological options help manage diabetes; increasing insulin secretion, improving insulin action, and reducing glucose absorption [7]. The more commonly used medications to lower blood glucose include; sulfonylureas, biguanides, meglitinides, alpha-glucosidase inhibitors, and thiazolidinediones (TZDs) [37]. Sulfonylureas and meglitinides stimulate pancreatic β-cells to release more insulin. Biguanides lower blood glucose by decreasing hepatic gluconeogenesis. Alpha-glucosidase inhibitors block the breakdown of starches in the intestine slowing the rise in blood glucose after a meal. TZDs assist insulin action in the muscle and fat while also reducing hepatic gluconeogenesis [37].

Sulfonylureas and meglitinides are insulinotropic agents that are considered a first-line treatment for individuals that are not overweight (BMI< 25 kg/m^2) [38]. Sulfonylureas increase cytosolic free calcium, a second messenger that stimulates insulin release from the β-cell of the pancreas [39]. These medications are taken before meals to stimulate the release of insulin for maintaining blood glucose. Since these treatment options stimulate the uptake of glucose it is possible it may cause hypoglycemia [37]. Data on the actual incidence of hypoglycemia in individuals with T2D using either sulfonylureas or meglitindies is scarce, but the risk is still present [40].

Metformin is a biguanide that is widely prescribed for T2D. It lowers blood glucose by decreasing fasting hepatic gluconeogenesis. Biguanides have been identified as a new class of complex I and adenosine triphosphate (ATP) synthase inhibitors [41]. Metformin inhibits phosphorylation of targets of protein kinase A, which blocks glucagon-dependent glucose output from hepatocytes [42]. Biguanides also make skeletal muscle more insulin sensitive by activating AMP-activated protein kinase (AMPK), which is important in insulin signaling and increases glucose uptake (ADA). Metformin is a first-choice treatment for overweight individuals (BMI> 25 kg/m^2) [38].

Alpha-glucosidase inhibitors are taken with meals and also a first-line treatment recommendation for overweight (BMI> 25 kg/m^2) individuals [37]. Alpha-glucosidase inhibitors interfere with the last step in carbohydrate digestion, conversion of disaccharides to monosaccharides in the gastrointestinal tract, which decreases absorption of glucose [43].

TZDs are the most popular class of oral drugs used to improve insulin sensitivity. They accounts for up to 20 percent of the drugs prescribed to diabetics in the United States [44]. An advantage of using TZDs is that they do not induce hypoglycemia as some comparable drugs like sulfonylureas [45]. TZDs mimic or enhance certain actions of insulin on carbohydrate and lipid metabolism, which result in antihyperglycemic effects [46]. TZDs increase fatty acid uptake and lipogenesis in adipose tissue [46]. Though TZDs do not modulate insulin secretion in β-cells, it increases insulin sensitivity in hepatic and adipose tissue [47]. TZDs stimulate transcriptional factors in adipose tissue by activating the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) [46]. PPARs are ligand-regulated transcription factors that control gene expression by binding to the specific response elements in promoter region of DNA. When PPARs obligate as heterodimers with a retinoid X receptor (RXR), this interaction while still ligand dependent increases the rate of transcription of PPAR target genes involved in glucose and lipid metabolism [48].

PPARs play a role as lipid sensors and regulators of lipid metabolism [48]. Activation of PPARγ, by ligands such as TZDs dramatically improves peripheral insulin sensitivity and reduces plasma glucose concentrations [49]. Though the exact mechanism is not fully understood, there are several possibilities as to how this drug improves peripheral insulin sensitivity and reduces plasma glucose concentrations. First, the benefits might be due to adipocyte differentiation where TZDs increase fat-cell number (hyperplasia), allowing for greater lipid storage capacity, which increases protection of non-adipose tissues such as muscle, liver, and pancreatic islets from excess lipid accumulation [49]. Second, PPARγ agonists might act on mature adipocytes altering the

production of adipose-derived hormones or metabolic signals functioning to improve metabolic parameters in tissues and organs like muscle, liver, and pancreas [49].

Rosiglitazone and pioglitazone are two drugs classified as TZDs. They are used in the treatment of T2D as monotherapy or combination therapy [50]. In addition to decreasing blood glucose values, these drugs are also beneficial for cardiovascular parameters such as lipids, blood pressure, inflammatory biomarkers and endothelial function [50]. However, there is evidence that these drugs can also contribute to edema, which can aggravate cardiovascular disease [50]. Individuals with T2D are already at an increased risk for developing cardiovascular disease, which makes the use of TZDs, like rosiglitazone and pioglitazone, a challenge in the treatment of T2D. Though the use of TZDs like rosiglitazone and pioglitazone are effective in lowering blood glucose their side effects prove that other treatment options need to be explored.

Fruit and vegetables and their glucose-lowering properties

Dietary strategies to alleviate metabolic complications of obesity are being investigated as complementary or even alternatives to pharmaceutical interventions [51]. Fruits and vegetables have been found to possess many protective health benefits including lowering blood glucose and improving lipid profiles.

Whole blueberry powder added to a high-fat diet was found to inhibit early inflammatory events in adipose tissue protecting against obesity-associated insulin resistance [51]. Improvement in intraperitoneal insulin tolerance test was also observed in the blueberry supplement group compared to the high fat control group. Additionally, the blueberry powder group showed increased skeletal muscle glucose uptake and decreased hepatic glucose production. These findings demonstrate that whole blueberry powder can improve glycaemia in obesity-induced insulin resistance [51].

In addition to blueberries, raisins have been found to possess anti-hyperglycemic properties. Corinthian raisins are produced in Southern Greece and are high in fiber, complex carbohydrates, vitamins and minerals, and polyphenols, which have antioxidant properties and can influence intestinal glucose uptake [52]. Healthy individuals or individuals diagnosed as having T2D given Corinthian raisins showed decreased glucose and insulin responses [52]. Corinthian raisins exhibit a lower glycemic index and are an appropriate food for individuals with diabetes [52].

Apples are the second most popular fruit consumed in the United States and also possess many health benefits including anti-hyperglycemic effect [53]. High antioxidant and phenolic phytochemical compounds contribute to apples potential for reducing chronic diseases like T2D [53]. Quercetin, a major component of the peel of an apple is a constituent associated with decreased risk for T2D [53]. However, growing conditions, variety, and part of the fruit affects the kind and amount of bioactive compounds found in the fruit. A study by Barbosa and colleagues compared ten different apple varieties to measure their phenolic and antioxidant capacity [53]. The peel of an apple has been reported to have a high antioxidant capacity, in fact, a whole apple (about 200g) can provide on average up to 40-50 mg total soluble phenolics, 25% of that from the peel [53]. The studies previously discussed show that fruits, because of their antioxidant and phenolic components can have glucose-lowering capabilities.

Studies on mango

Mango is another fruit with abundant antioxidant and phenolic components, which make it a potential natural product able to provide a protective role in modulating blood glucose and other clinical parameters [19]. A study by Lucas and colleagues investigated the effects of freeze-dried mango pulp on adiposity, glucose metabolism, and lipid profiles in mice fed a high fat diet. The effect of mango was also compared to the hypolipodemic drug (fenofibrate) and

hypoglycemic drug (rosiglitazone). This study found that mango was comparable to fenofibrate and rosiglitazone in preventing increases in fat mass and percent body fat and also lowered insulin resistance [19]. Perpetuo and colleagues investigated the effects of three different doses of mango flour (5,10, and 15%) on normal and diabetic rats [54]. They found that all three doses of mango significantly reduced glucose levels in diabetic rats with 30 days of treatment [54]. They did a follow up study and found that 5% mango flour supplementation over 90 days significantly reduced glucose levels and increased hepatic glycogen in diabetic rats [54].

Extract of the different parts of the mango plant have been investigated for their ability to lower blood glucose parameters. Muruganandan *et al*. used an animal model to investigate the effects of mango leaf extract rich in mangiferin in an animal model of type 1 diabetes, the streptozotocin (STZ)-induced diabetic rats [14]. Mango leaf extract was shown to be as effective as insulin at lowering blood glucose, triglycerides, total cholesterol, LDL cholesterol, and increasing HDL cholesterol after 28 days of supplementation in STZ-induced diabetic rats [14]. Aqueous mangiferin leaf extract was also decreased the progressive rise in glucose during a glucose tolerance challenge in STZ-induced diabetic rats [55]. A study by Zhang and colleagues looked at the effects of mango leaf extract on glucose and triglyceride levels in mice. They found that mango leaves extract significantly reduced serum glucose and lipid concentrations [56]. Protein and mRNA expression of GLUT4 was up-regulated in the group treated with mango leaves extract compared to the control group [56]. Saleh and colleagues also investigated the effects of mango bark extract rich in mangiferin supplementation on STZ-induced diabetic rats. After 28 days, the mango bark extract decreased blood glucose, HOMA-IR, triglycerides, cholesterol, and TNF-α levels, while also increasing β-cell function and adiponectin levels [57].

The glucose lowering ability of mango has been compared to other fruits as well in clinical trials. A clinical study assessed postprandial glucose response of five fruits, banana, orange, pineapple, pawpaw, and mango, compared to a glucose solution in T2D subjects [58]. The

results of this study showed that banana, orange, pineapple, mango and pawpaw had similar postprandial glucose values which were lower than those of a post-glucose load [58]. The plasma glucose response of mango, pawpaw and orange were consistently lower, but not significantly, than banana and pineapple [58]. The consistently favorable glycemic responses of mango make it a recommendable choice of fruit for diabetic individuals to consume [58]. Another study showed that 208.5 g mango lowered the postprandial glucose and insulin responses of ten diabetic female subjects when compared to their responses to banana, durian, pineapple and rambutan [59]. Robels-Sanchez and colleagues looked at the effects of mango in normolipidemic subjects [60]. They divided the subjects into two groups, one consuming whole mango (including peel and seed) for 30 days and the other consuming fresh-cut mango (excluding peel and seed) for 30 days. Both types of mango had significant effects on plasma antioxidant capacity and triacylglycerides [60]. These findings indicate mango as a component of a healthy diet for hypertriacylglyceridemic prevention [60]. Evans *et al*. found that after twelve weeks of supplementation mango significantly reduced fasting blood glucose in obese subjects and increased circulating insulin levels in male obese subjects [61]. The animal and human studies discussed above demonstrates the hypoglycemic properties of mango, as well as its effects in improving common health complications associated with diabetes such as dyslipidemia.

CHAPTER III

METHODOLOGY

Study participants

Male and female adults between 18-70 years of age with mild to moderate hyperglycemia (i.e. fasting blood glucose $\geq 100 \leq 126$ mg/dL) and currently not taking glucose-lowering medications with normal liver and kidney function test were recruited in the study. Individuals having any form of pre-existing disease (e.g., cancer, heart disease, etc.), or liver or renal disorders, who are pregnant or lactating, consuming mega-doses of antioxidants and/or fish oil supplements (>1g/day), abnormal hemoglobin concentrations (normal range: 12.0-18.0 g/dL), white blood cell count (normal range: 4.0-11.0 K/mm^3), platelets (140-440 K/mm^3), and liver enzymes (normal range for aspartate aminotransferase, AST: 7-40 units/L; alanine aminotransferase, ALT: 10-45 units/L), who smoke or use any form of tobacco, and consume more than 1 oz. of alcohol per day were excluded from this study.

Study supplements

Mango and placebo supplements were prepared on the campus of Oklahoma State University. Ripe Tommy Atkins variety mango was purchased from a local market, freeze-dried,

ground into a powder, and weighed into an opaque plastic bag (10g/bag). The dose of freeze-dried mango powder (10g/d) and the variety were based on an animal study that found 1% mango to be effective at improving blood glucose and clinical parameters of mice fed a high fat diet and our recent human study [19, 61]. The macronutrient composition of mango supplement used in the study is shown in Table 1. The placebo was prepared using yellow colored sugar, fiber (FiberStir LLC, Plymouth, MN) and powdered milk. The placebo supplement was adjusted to have similar carbohydrate, fat, protein and fiber content to that of the mango supplement and weighed into similar bags as the mango supplement.

Table 1. Nutrient composition of the mango supplement

Nutrients	**Amount per bag (10g freeze-dried mango)**
Carbohydrate (g)	8.58
Protein (g)	0.501
Fat (g)	0.175
Fiber (g)	1.29
Calcium (g)	7.92
Phosphorus (mg)	12.3
Calories (kcal)	37.9

Nutrient composition was analyzed by NP Analytical (St. Louis, MO)

Study design and visits

A cross-over study design was used. Participants were asked to consume the pre-weighed, freeze-dried mango or placebo daily for 12 weeks with 3-4 weeks wash-out between treatments. No stipulations were given on how the supplement was to be consumed. Participants were also asked to maintain their normal diet, exercise, and lifestyle habits throughout the entire study. Study protocols were reviewed and approved by Institutional Review Board at Oklahoma State University (IRB Number HE.11.41).

After an initial telephone pre-screening, participants came to the study site and fasting blood glucose was measured with a home glucometer testing kit (Onetouch, Shelton, CT). To qualify for the study fasting blood glucose should be between 100mg/dL and 125 mg/dL. If participants qualified, they were asked to come to the Stillwater Medical Center (Stillwater, OK) for a fasting blood draw. A summary of procedures for each visit is shown in Table 2.

Table 2. Procedures performed during the study

Procedures	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**
Finger stick blood glucose measurement	x						
Explain the study protocol and get consent	x						
Medical history questionnaire	x						
Anthropometric measurement	x	x	x	x	x	x	x
Blood pressure measurements	x	x	x	x	x	x	x
Physical activity questionnaire	x		x	x	x	x	x
3 day food record							
Given	x	x	x	x	x	x	
Collected		x	x	x	x	x	x
Fasting blood collection		x	x	x	x	x	x
Oral glucose tolerance test (OGTT)				x			x
Urine pregnancy test (women participants of child-bearing age)		x		x	x		x
Body composition by dual energy xray absorptiometry (DXA)		x		x	x		x
Treatment assignment		x			x		

Supplements							
Given		x	x		x	x	
Collected			x	x		x	x
Calendar							
Given		x	x		x	x	
Collected			x	x		x	x
Honorarium given		x	x	x	x	x	x

Questionnaires

A medical history questionnaire was obtained from all study participants to ensure that all inclusion and exclusion criteria were met. A physical activity questionnaire was collected from all subjects at each visit to determine exercise habits and to make sure that they maintained these activities throughout the duration of the study. The physical activity questionnaire was based on the method of Anderson [62]. This questionnaire breaks up daily physical activity into nine different categories: sleep, sedentary work, standing/walking, heavy physical work, biking or walking to work, leisure time, light activity, moderate activity, and strenuous activity. These categories describe the total amount of physical activity in an average week, which was reported in minutes per day or hours per week depending on the specific question. A 3-day food-record was collected at each visit to determine the participant's dietary habits throughout the study. Nutrient intake was analyzed using the Diet Analysis Plus Software (Cengage, Farmington Hills, MI).

Anthropometric measures and body composition

Height, weight, blood pressure, hip circumference, and waist circumference were measured at each visit. Height was measured using a Shorr Board stadiometer (Shorr Productions, Olney, MD), and weight using a digital wheelchair scale (Seca Southwest Scales, Amarillo, TX). After participants relaxed for a few minutes in a sitting position, blood pressure was measured twice using a digital blood pressure monitor (ReliOn, Oncue HealthCare Inc, Bennockburn, IL). Hip and waist circumference was measured using a standard measuring tape (Creative Health

Products, Ann Arbor, MI). The waist circumference was defined as the midpoint between the highest point of the iliac crest and the inferior portion of the costal margin in the mid-axillary plane. The hip circumference was determined at the level of the maximum posterior extension of the buttocks.

Body composition measures were assessed at baseline and at the end of 3-months of each dietary supplementation using a dual-energy X-ray absorptiometry (DXA; Hologic, Bedford, MA). Subjects were scanned while lying in a supine position with arms at their sides. Measurements of body composition included percent fat mass, lean mass, and bone mineral content (BMC).

Blood collection and oral glucose tolerance test (OGTT)

Fasting blood sample was drawn from the vein in the right or left arm by a licensed phlebotomist or a registered nurse at Stillwater Medical Center (Stillwater, OK) into a plasma or serum tube. Hepatic function, complete blood count, lipid profiles, glucose, glycated hemoglobin analyses were performed at Stillwater Medical Center. A portion of blood samples were taken to the Nutritional Sciences Department (Oklahoma State University, Stillwater OK), centrifuged at 3200 x g for 20 minutes at 4° C to obtain serum and plasma, aliquoted, and stored at -80 ^{o}C until further analyses.

The OGTT was administered at the end of each dietary treatment at Stillwater Medical Center. After the baseline blood draw, participants were asked to drink a 75 g oral glucose solution within 5 minutes (Azer Scientific, Morgantown, PA). Blood samples were taken 2 hours after drinking the glucose solution and blood glucose was assessed at Stillwater Medical Center.

Plasma insulin, leptin, and adiponectin

Enzyme linked immunosorbent assay (ELISA) kits from EMD Millipore Corporation (St. Charles, MO) were used to determine plasma concentrations of insulin, adiponectin, and leptin. With ELISA, antigens from the sample react to the capture antibody on the surface of a 96-well plate. A specific detection antibody was added to the well and binds to antibody-antigen complex, which is then labeled with horseradish peroxidase followed by the substrate 3,3',5,5'-tetramethylbenzidine forming a colored solution. Absorbance was measured using a microplate reader (Biotek, Winooski, VT) and is directly proportional to the concentration of captured insulin, leptin, or adiponectin in the unknown plasma sample.

Serum thiobarbituric acid reactive substances (TBARS) and total antioxidant

Assay kits from Cayman Chemical Company (Ann Arbor, MI) were used to determine serum concentrations of TBARS and total antioxidants. Cellular oxidative stress results in the formation of reactive and unstable lipid hydroperoxides. The decomposition of these unstable peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehydes (MDA), which can be quantified colorimetrically following its reaction with TBARS (Cayman Chemical Company, Ann Arbor, MI). This measurement is a well-established method for screening and monitoring lipid peroxidation. The assay of TBARS measures MDA reaction with TBA at high temperatures and data is collected colorimetrically at 530nm and 550nm.

Total antioxidants activity is the sum of endogenous and food-derived antioxidants. Cooperation of all the antioxidants provides the greatest protection against reactive oxygen or nitrogen radicals rather than any single compound alone. Therefore, the overall antioxidant capacity can give more relevant biological information compared to the results of an individual component, because it considers the cumulative effect of all the antioxidants that are present (Cayman Chemical Company, Ann Arbor, MI). The assay for total antioxidant allows the

antioxidants in the sample to inhibit the oxidation of 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) by metmyoglobin. The amount of ABTS produced was determined by reading the absorbance of 750 nm or 450 nm. Under the reaction conditions, the antioxidant in the sample will suppress the absorbance to a degree that is proportional to their concentration. (Cayman Chemical Company, Ann Arbor, MI).

Cytokines and diabetes markers analysis using Bio-plex

Bioplex Pro cytokine and diabetes assay kits from Bio-Rad Laboratories (Hercules, CA) were used to measure twenty-seven different cytokines interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17A (IL-17A), eotaxin, basic fibroblast growth factor (basic FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1alpha (MIP-1α), macrophage inflammatory protein 1beta (MIP-1β), platelet-derived growth factor (PDGF), C-C motif chemokine 5 (CCL5) (RANTES), tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF)) and ten different diabetes markers (connecting peptide (C-peptide), ghrelin, gastric inhibitory peptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, insulin, leptin, plasminogen activator inhibitor 1 (PAI-1), resistin, visfatin).

The Bio-Plex Pro assay quantifies multiple protein biomarkers in a single well of a 96-well plate. This assay uses capture antibodies that are directed against the desired biomarkers and are covalently coupled to magnetic beads. The capture antibody attached to the magnetic beads reacts with the antigen in the sample. A biotinylated detection antibody was added to bind to the

antibody-antigen complex attached to the magnetic bead and then labeled with a final detection complex formed by the addition of streptavidin-phycoerythrin (SA-PE) conjugate. The phycoerythrin serves as a fluorescent indicator and the beads containing the complex were read in a Bio-Plex MAGPIX Multiplex Reader and concentration was calculated based on a standard curve (Bio-Rad Laboratories, Inc. Hercules, CA).

Statistical analyses

All values are presented as mean ± standard deviation. Statistical data were computed and analyzed by one-way ANOVA using SAS software version 9.3 (SAS Institute, Cary, NC). The general linear model (GLM) procedure was used to calculate variance and least square means, while the means were compared using Fisher's least significant difference. Data are considered significantly different with P value <0.05.

CHAPTER IV

RESULTS

Baseline characteristics

The baseline characteristics of the participants are shown in Table 3. Twenty-eight individuals with slightly elevated blood glucose participated in the three month study. All screening blood glucose obtained using a home testing glucometer (Onetouch, Shelton, CT) was 103.4 ± 3.7, and 106.9 ± 8.2 mg/dL for the mango and placebo treatments respectively. Twenty-two individuals completed both arms of the study while six individuals finished only one arm of the study (four individuals only took the mango supplement while another two individuals took only the placebo). Therefore, twenty-six and twenty-four individuals received the mango and placebo supplement, respectively. The major reason for dropping out of the study and not crossing-over to the other treatment was time constraints, and one study participant had an allergic reaction to the mango supplement.

The mean age of the subjects participants were 38.8 ± 14.1 and 44.0 ± 15.4 years for the mango and placebo groups, respectively (Table 3). The oldest study participant was seventy years old and the youngest was eighteen years old. Ten male and sixteen female participants took the mango supplement while seven male and seventeen female participants took the placebo supplement. There were no differences in weight, height, and BMI at baseline among the two groups.

Food intake and physical activity

There were no significant differences found in food intake of the subjects on either supplement before or after supplementation. The mean total caloric intake of the study participants for both groups during the entire study was around 1800 kcal (Table 4). Vitamin C intake tended (P=0.0615) to increase for the mango group after 12 weeks of supplementation (Table 4).

There was no significant difference in the amount of sleep and physical activity between the two groups before and after supplementation. The study participants spend about 400 minutes of sleep per week (Table 5). They also spend about 170 minutes of moderate activity per week (Table 5).

Anthropometrics, body composition, and lipids and hepatic panel

There were no significant differences between the two groups of baseline blood pressure, waist and hip circumference, and BMI. After 90 days of treatment there were no significant differences in weight, systolic blood pressure, diastolic blood pressure, BMI, and waist or hip circumference between the mango and the placebo groups (Table 6). There were also no significant differences in whole body area, bone mineral content (BMC), bone mineral density (BMD), fat mass, or percent body fat with mango supplementation when compared to the control (Table 6). Additionally, when these variables were examined for differences due to time, there were no differences between the two groups when comparing the change from baseline with theses parameters.

There was no significant difference between mango and placebo as well as change from baseline in lipid profile assessed by measuring total cholesterol, triglyceride, LDL, HDL, VLDL cholesterol, and LDL/HDL (Table 7). No significant differences were observed with treatment and time when measuring serum total protein, albumin, and globulin to monitor possible diabetic

nephropathy (Table 5). Hepatic function was assessed by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase and there were no differences with treatment and time in these parameters (Table 7). Bilirubin, which is associated with a lower prevalence of oxidative stress-mediated diseases like T2D, significantly increased (P=0.0017) for both treatment groups over the 90 day study (Table 7).

Diabetes parameters, cytokines, and lipid peroxidation

There was no significant difference in fasting blood glucose, HbA1C%, insulin, HOMA-IR or blood glucose after two hours of glucose tolerance test with mango supplementation compared to the control. There was also no difference between the two treatments when comparing change from baseline (Table 8).

Ghrelin, the hunger hormone, was significantly lower (P=0.0130) in the mango group compared to the control but there was no significant difference with change from baseline (Table 9). There were no significant differences in C-peptide, GIP, GLP-1, and visfatin (an indicator of β-cell function). Glucagon, an antagonist of insulin was also found to not be significant with treatment and time. Four markers of obesity and weight control; leptin, adiponectin, PAI-1 and resistin were measured and there were no significant differences with mango supplementation compared to the control as well as no change with time (Table9).

IL-13 an anti-inflammatory protein was significantly lower (P=0.0284) in the mango group when compared to the control, but there was no difference in change from baseline. The pro-inflammatory protein, TNF-α, was significantly lower (P=0.0399) in the mango group when compared to the control, but similar to the IL-13 was not changed with time (Table 10). Antioxidants were measured to assess oxidative stress and were found to be significantly lower (P=0.0371) in the mango group when compared to the control group (Table 10). TBARS was also measured to assess lipid peroxidation and there were no significant differences. There was no

significant difference in pro-inflammatory cytokines, IL-1β, IL-8, IL-12p70, IFNγ, IP-10, MCP-1, MIP-1α ,MIP-1β, or RANTES. Anti-inflammatory proteins, IL-1ra, IL-6 and IL-10 displayed no significant differences. Immunity was assessed by measuring IL-2, IL-4, IL-5, and IL-7, and there were no significant differences found. There were no significant differences when measuring IL-9, FGF, GCSF, PDGF-BB, and VEGF to assess cell growth (Table 10).

Table 3. Baseline characteristics

Parameter	Mango (n=26)	Placebo (n=24)	P value
No. of male (female) participants	10 (16)	7 (17)	
Age *(yrs)*	38.8 ± 14.1	44.0 ± 15.4	0.3712
Weight *(lbs)*	188.6± 32.8	196.5± 40.6	0.5810
Height (*in*)	65.8 ± 3.4	66.0 ± 3.4	0.8804
BMI (kg/m^2)	30.8± 6.9	31.9± 6.2	0.6695
Screening blood glucose (*mg/dL*)	103.4± 3.7	106.9± 8.2	0.1734

Values are mean ± standard deviation. Significance level set at *P* value <0.05. Body mass index (BMI); screening blood glucose was assessed by a home-testing glucometer (Onetouch, Shelton, CT).

Table 4. Effect of 90 day supplementation with mango or a placebo on dietary intake of individuals with moderately elevated blood glucose

Parameter	Mango			Placebo			P value			P value
	Baseline (n=17)	Final (n=20)	Change§	Baseline (n=18)	Final (n=18)	Change§	Time	Treat-ment	Time X Treat-ment	change from baseline
Total Energy (kcal)	1858.2± 865.6	1728.2± 766.3	130.0± 358.4	1762.3± 546.2	1845.5± 875.2	-83.1± 996.0	0.9057	0.9568	0.5902	0.4406
Macronutrients										
Protein (g)	75.3± 24.3	66.4± 19.0	9.0± 16.3	67.8± 20.5	74.3± 22.6	-6.5± 30.8	0.8276	0.9683	0.1673	0.0962
Carbohydrate (g)	224.4± 117.7	219.0± 94.3	5.4± 51.5	228.1± 76.1	245.6± 151.7	-17.5± 167.4	0.8357	0.6020	0.6940	0.6165
Sugar (*g*)	92.5± 50.1	77.1± 42.3	15.4± 28.7	82.7± 41.0	80.2± 54.3	2.5± 47.9	0.4599	0.7828	0.5962	0.3766
Fiber (*g*)	16.4± 8.7	15.9± 10.8	0.9± 5.1	18.4± 6.6	18.3± 15.2	0.1± 16.5	0.8664	0.4684	0.8853	0.8577
Lipids										
Total Fat (g)	73.3± 38.3	65.1± 42.4	8.3± 23.6	66.0± 25.5	63.8± 33.2	2.3± 32.1	0.5581	0.6317	0.7401	0.5619
Saturated Fat (g)	24.2± 12.4	21.4± 13.1	2.8± 9.0	21.3± 9.3	20.8± 11.6	0.5± 10.4	0.5822	0.5604	0.6998	0.5188
MUFAs (g)	19.4± 11.5	17.0± 11.7	2.5± 11.2	18.8± 9.6	16.9± 10.2	1.8± 12.6	0.4264	0.9089	0.9096	0.8851
PUFAs (g)	11.8±12.2	11.6± 11.0	0.2± 9.2	10.6± 5.7	10.9± 10.5	-0.4± 12.9	0.9714	0.7220	0.9162	0.8945
Trans fatty acid (*g*)	0.4± 0.7	0.7± 0.8	-0.2± 0.8	0.5± 0.5	0.4± 0.4	0.1± 0.5	0.7449	0.6021	0.2630	0.1478
Cholesterol (*mg*)	215.1± 112.9	211.5± 114.6	3.6± 145.1	195.6± 86.5	237.0±178.2	-41.4± 191.0	0.5633	0.9260	0.4918	0.4680
Omega 6 (*g*)	9.1± 11.1	9.6± 10.0	-0.6±8.2	8.3± 5.2	8.9 ± 10.0	-0.6± 12.0	0.8100	0.7527	0.9959	0.9948
Omega 3 (*g*)	0.8± 0.9	0.8± 0.7	-0.0± 0.9	0.9± 0.6	0.9± 0.9	-0.0± 1.2	0.9985	0.6499	0.9667	0.9659
Vitamins										
Thiamin (*mg*)	1.0± 0.6	1.2± 0.5	-0.2±0.5	1.1± 0.4	1.3± 1.0	-0.2± 1.1	0.2526	0.5375	0.8294	0.8245
Riboflavin (*mg*)	1.3± 0.8	1.4± 0.7	0.06± 0.7	1.3± 0.7	1.7± 1.2	-0.4± 1.4	0.2776	0.3999	0.4258	0.3726
Niacin (*mg*)	16.4± 9.5	16.7± 6.6	-0.3± 6.4	17.7± 7.4	21.2± 13.4	-3.5± 16.5	0.4424	0.2444	0.5122	0.4837
Vitamin B12 (*mg*)	3.2± 3.3	2.8± 2.2	0.5± 2.2	4.4± 6.3	7.1± 16.9	-2.7± 17.9	0.6526	0.2634	0.4973	0.4914

Table 4. Cont.

Parameter	Mango			Placebo			P value			P value
	Baseline (n=17)	Final (n=20)	Change§	Baseline (n=18)	Final (n=18)	Change§	Time	Treat-ment	Time X Treat-ment	change from baseline
Vitamin C (*mg*)	56.5± 46.8	118.2± 131.2	-61.6± 127.1	81.4± 53.7	75.1± 52.7	6.3± 56.1	0.1707	0.6495	0.0939	0.0615
Vitamin E (*mg*)	5.1± 5.7	5.6± 4.9	-0.6±3.8	5.9± 4.9	6.7± 5.8	-0.8± 4.4	0.6016	0.5010	0.9200	0.8534
Minerals										
Calcium (*mg*)	712.2± 414.1	683.5± 333.6	28.6± 291.3	704.6± 228.9	759.9± 382.3	-55.2± 433.1	0.8804	0.6973	0.6353	0.5351
Iron (*mg*)	12.7± 6.7	15.2± 7.0	-2.5±7.2	15.1± 5.8	15.0± 9.6	-0.2± 10.4	0.5188	0.5507	0.4932	0.4049
Magnesium (*mg*)	201.7± 138.3	186.5± 118.9	15.2± 52.1	196.6±82.3	230.8± 189.9	-35.7± 198.5	0.7875	0.5801	0.4848	0.4122
Potassium (*mg*)	1800.8± 1054.6	1727.4± 863.7	73.4±744.9	1894.8± 726.1	1951.2± 937.0	-56.4± 1193.7	0.9705	0.4905	0.7778	0.7211
Zinc (*mg*)	7.1± 4.1	7.3± 4.2	-0.2±4.5	7.9± 4.0	7.9± 4.1	-0.0± 5.4	0.8952	0.5281	0.9327	0.9223
Sodium (*mg*)	2790.4±1493.0	2947.5±1218.3	-157.1± 846.6	2894.4± 1112.1	2828.44± 1639.6	66.0± 1776.7	0.8973	0.9831	0.7521	0.6623

Values are mean ± standard deviation. Significance level set at *P* value <0.05. monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs). § Change means baseline minus final values.

Table 5. Effect of 90 day supplementation with mango or a placebo on physical activity of individuals with moderately elevated blood glucose

Parameter	**Mango**			**Placebo**			**P value**			**P value**
	Baseline (n=24)	Final (n=25)	Change§	Baseline (n=24)	Final (n=23)	Change§	Time	Treatment	Time x Treat-ment	change from baseline
Physical Activity (*minutes*)										
Daily Sleep	429± 69.6	426± 40.4	3.0± 65.2	435± 89.0	418± 47.4	17.3± 78.1	0.4560	0.9500	0.5921	1.0
Daily Sedentary Work	316± 186.4	351± 169.6	-35.0± 189.2	335± 189.7	369± 163.9	-34.2± 204.7	0.3455	0.6246	0.9935	0.1549
Daily Standing/Walking	160± 147.6	149± 149.3	11.0± 186.3	142± 153.1	191± 265.6	-49.1± 276.5	0.6139	0.7501	0.4304	0.5942
Daily Heavy Physical Work	62± 119.7	62± 119.7	0.0± 123.8	73± 134.0	98± 198.0	-24.9± 116.8	0.6821	0.4334	0.6740	0.8284
Daily Biking/walk to work	22± 311.3	47± 85.3	-26.1± 89.0	30± 63.4	38± 98.7	-8.2± 120.3	0.2724	0.9710	0.5592	0.6178
Daily Leisure Time	158± 124.1	146± 61.4	12.3± 118.0	158± 100.3	125± 69.3	32.8± 96.5	0.2324	0.5863	0.5638	0.8864
Weekly Light Activity	350± 379.2	223±173.7	127.0± 401.3	301± 365.3	394± 555.4	-93.1± 571.6	0.8344	0.4408	0.1691	0.2518
Weekly Moderate activity	234± 485.5	145± 147.3	88.9± 536.5	184± 244.6	159± 156.2	25.2± 267.3	0.3455	0.7639	0.6004	0.5868
Weekly Strenuous activity	106± 168.3	111± 151.1	-5.5± 173.1	85± 157.4	135.2± 190.1	-50.4± 191.5	0.4230	0.9685	0.5115	0.3939

Values are mean ± standard deviation. Significance level set at *P* value <0.05. § Change means baseline minus final values.

Table 6. Effects of 90 days supplementation with mango or a placebo on weight, blood pressure, waist and hip circumference, and body composition in individuals with moderately elevated blood glucose

Parameter	Mango			Placebo			P value			P value
	Baseline (n=26)	Final (n=26)	Change§	Baseline (n=24)	Final (n=24)	Change§	Time	Treatment	Time X Treatment	change from baseline
Weight *(lbs)*	186.7± 9.1	193.3± 39.3	-6.6± 21.8	189.3± 38.4	190.9± 40.0	-1.6± 6.0	0.5871	0.9753	0.7446	0.1515
SBP *(mm Hg)*	128.3± 15.3	129.6± 15.9	-1.3± 18.2	127.5± 13.0	130.9± 12.9	-3.4± 10.4	0.4217	0.9140	0.7111	0.6276
DBP *(mm Hg)*	80.2± 12.8	79.5± 11.6	0.7± 11.9	81.2± 10.7	79.7± 9.9	1.5± 6.0	0.6290	0.7877	0.8687	0.8537
BMI *(kg/m²)*	31.0± 6.6	31.5± 6.9	-0.5± 0.7	30.8± 6.4	31.3± 6.5	-0.5± 4.9	0.7296	0.8693	0.9931	0.2432
Waist circumference *(in)**	39.5± 5.6	40.3± 5.8	-0.8± 12.8	40.0± 5.7	39.7± 6.2	0.3± 17.7	0.8604	0.9620	0.6848	0.6972
Hip circumference *(in)**	43.5± 5.4	44.24± 5.3	-0.7± 142	44.1± 5.7	44.5± 6.3	-0.4± 17.6	0.6509	0.7515	0.8939	0.6810
Body Composition										
Area *(cm²)*	2072.8± 181.3	2086.8± 193.2	-14.0± 28.5	2061.5± 194.5	2063.1± 190.6	-1.6± 32.0	0.8374	0.6470	0.8711	0.3374
BMC *(g)*	2447.7± 361.0	2587.7± 766.0	-140± 614.2	2397.8± 334.9	2407.5± 325.7	-9.7± 47.1	0.4450	0.2411	0.5059	0.3460
BMD *(g/cm²)*	1.17± 0.1	1.18± 0.1	-0.0± 0.0	1.16± 0.01	1.16± 0.01	-0.00± 0.0	0.9035	0.4621	0.9530	0.4458
Fat Mass *(kg)*	30.85± 12.36	30.28± 11.10	0.58± 1.43	31.87± 12.40	31.95± 12.38	-0.76± 1.74	0.9189	0.5841	0.8944	0.7156
% Fat	35.0± 9.5	34.4± 8.6	0.6± 1.5	36.1± 9.2	36.1± 9.3	0.0± 1.4	0.8560	0.4375	0.8631	0.9768

Values are mean ± standard deviation. Significance level set at *P* value <0.05. systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), body mass composition (BMC), bone mineral density (BMD).* n= 18 placebo base, 19 placebo final; 21 mango base, 24 mango final. § Change means baseline minus final values.

Table 7. Effects of 90 days supplementation with mango or a placebo on lipids and liver panel in individuals with moderately elevated blood glucose

Parameters	Mango			Placebo			P value			P value
	Baseline (n=26)	Final (n=26)	Change§	Baseline (n=24)	Final (n=24)	Change§	Time	Treatment	Time X Treat-ment	change from baseline
Lipid Panel										
Total Cholesterol *(mg/dL)*	178.2± 30.7	180.7± 30.8	-2.3± 20.3	181.2± 29.9	178.0± 29.4	3.2± 18.6	0.9281	0.7344	0.7728	0.4914
Triglycerides *(mg/dL)*	125.8± 85.2	134.3± 76.8	-9.0± 54.8	127.6± 77.8	117.8± 53.5	9.8± 30.4	0.8929	0.8228	0.6639	0.5093
LDL Cholesterol *(mg/dL)*	106.6± 26.5	107.4± 25.6	-0.1± 16.0	105.8± 26.4	103.0± 25.5	2.1± 14.6	0.9881	0.9334	0.8871	0.6221
HDL Cholesterol *(mg/dL)*	48.0± 11.3	46.6± 10.3	1.4± 7.3	50.1± 11.4	51.3± 10.0	-1.2± 6.9	0.8094	0.2757	0.7097	0.4211
VLDL Cholesterol *(mg/dL)*	25.1± 17.1	26.8± 15.3	-1.8± 11.0	25.5± 15.8	23.5± 10.6	2.0± 6.1	0.8873	0.8286	0.6446	0.4684
LDL/HDL *(mg/dL)*	2.4± 1.0	2.4± 0.9	-0.0± 0.4	2.2± 0.9	2.1± 0.7	0.1± 0.4	0.9453	0.4941	0.7863	0.2431
Hepatic Panel										
Total protein (*gm/dL*)	7.1± 0.5	7.2± 0.5	-0.1± 0.5	7.1± 0.4	7.1± 0.5	0.0± 0.5	0.8182	0.8105	0.6950	0.6217
Albumin (*gm/dL*)	4.2± 0.4	4.2± 0.4	-0.0± 0.3	4.2± 0.3	4.1± 0.4	0.1± 0.4	0.9942	0.9354	0.9293	0.9600
Globulin (*gm/dL*)	2.3± 0.4	3.0± 0.4	-0.0± 0.3	2.96± 0.3	2.9± 0.3	0.0± 0.3	0.8405	0.7248	0.5551	0.4231
AST (*U/L*)	27.0± 16.1	26.5± 7.5	0.8± 13.5	27.9± 14.5	25.7± 9.7	2.2± 13.0	0.6019	0.9790	0.7509	0.6777
ALT(*U/L*)	39.6± 16.6	39.4± 12.1	0.7± 13.0	40.9± 27.9	36.3± 10.0	4.6± 28.5	0.5091	0.7970	0.5476	0.4421
Alkaline Phosphatase (*U/L*)	73.5± 17.2	76.7± 15.5	-2.1± 12.5	76.4± 21.8	78.9± 21.2	-2.5± 11.7	0.4622	0.5046	0.9293	0.9260
Bilirubin (*gm/dL*)	0.2± 0.2	0.3± 0.1	-0.1± 0.2	0.2± 0.1	0.3± 0.1	-0.1± 0.1	**0.0017**	0.8108	0.1588	0.1222

Values are mean ± standard deviation. Significance level set at *P* value <0.05. low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), aspartate aminotransferase (AST), alanine transaminase (ALT). § Change means baseline minus final values.

Table 8. Effects of 90 days supplementation with mango or a placebo on glucose parameters in individuals with moderately elevated blood glucose

Parameters	Mango			Placebo			P value			P value
	Baseline (n=26)	Final (n=26)	Change§	Baseline (n=24)	Final (n=24)	Change§	Time	Treatment	Time X Treat-ment	change from baseline
Glucose *(mg/dL)*	97.5± 9.4	95.5± 9.0	2.0± 7.5	97.8± 10.9	97.9± 10.7	0.1± 9.1	0.5953	0.5117	0.6381	0.4346
HbA1C %	5.4± 0.3	5.4± 0.3	-0.0± 0.3	5.4± 0.3	5.4± 0.3	-0.3± 1.2	0.3894	0.9487	0.9008	0.3076
Insulin *(mg/mL)*	6.45± 3.3	6.6± 3.4	-0.3± 2.0	6.2± 3.7	6.2± 4.1	-0.1± 1.8	0.9072	0.6977	0.9988	0.7707
HOMA-IR*(mg/mL)*	1.6± 0.9	1.6± 0.9	-0.0± 0.5	1.5± 1.1	1.6± 1.2	-0.0± 0.5	0.9646	0.8823	0.9143	0.9877
GTT2 hr *(mg/dL)**		107.6± 41.8			105.1± 42.1			0.8427		

Values are mean ± standard deviation. Significance level set at *P* value <0.05. glycated hemoglobin (HbA1C %), homeostatic model assessment-insulin resistance (HOMA-IR), 2 hour glucose tolerance test (GTT2 hr).*n= 26 and 22 individuals for the mango and placebo treatments, respectively. § Change means baseline minus final values.

Table 9. Effect of 90 day supplementation with mango or a placebo on plasma diabetes parameters in individuals with moderately elevated blood glucose

Parameter	Mango			Placebo			P value			P value
	Baseline (n=21)	Final (n=21)	Change[§]	Baseline (n=20)	Final (n=20)	Change[§]	Time	Treat-ment	Time X Treat-ment	change from baseline
C-Peptide *(pg/mL)*	1564.3± 651.7	1520.6± 667.2	43.7+ 184.0	1468.4± 636.8	1564.89± 710.1	-96.5+ 288.3	0.8583	0.8616	0.6355	0.0697
Ghrelin *(pg/mL)*	1272.6±520.7	1284.8±551.3	-12.2± 251.6	1628.6±736.2	1666.41±790.3	-37.8± 3338.4	0.8637	**0.0130**	0.9299	0.7841
Leptin *(mg/dL)**	16.0± 14.2	19.7± 18.3	-3.7± 8.8	18.1± 16.7	20.0± 22.6	-1.9± 12.4	0.9544	0.7404	0.9521	0.7412
Adiponectin *(µg/mL)***	9.3± 6.0	9.1± 5.3	0.2± 3.0	8.8± 6.5	9.0± 7.1	-0.1± 2.3	0.9906	0.8408	0.8829	0.4531
GIP *(pg/mL)*	565.2±314.5	551.8±291.1	13.4± 343.0	490.1±171.8	632.7±384.7	-142.7± 298.7	0.3335	0.2434	0.9653	0.1290
GLP-1 *(pg/mL)*	324.5±53.8	321.1±51.7	3.4± 41.9	302.1±37.9	306.1±51.9	-4.1± 45.4	0.9746	0.0901	0.7333	0.5873
Glucagon *(pg/mL)*	316.2±106.1	308.7±89.5	7.5± 40.1	331.9±108.8	344.7±122.7	-12.9 63.6	0.9107	0.2777	0.6689	0.2255
PAI-1 *(pg/mL)*	6350.8±2611.3	6498.5±2614.9	-147.8± 2230.0	6265.0±1959.7	6211.4±2358.9	53.6± 2410.8	0.9297	0.7268	0.8504	0.7827
Resistin *(pg/mL)*	3965.0±1169.1	4097.1±1234.3	-132.2± 571.4	4179.6±1297.1	4516.5±1519.3	-337.0± 656.7	0.4198	0.2764	0.7243	0.2927
Visfatin *(pg/mL)*	1148.0±720.6	959.8±619.2	188.3± 613.5	1145.2±636.4	889.5±631.6	255.7± 1046.0	0.1283	0.8010	0.8161	0.8014

Values are mean ± standard deviation. Significance level set at *P* value <0.05. connecting peptide (C-Peptide), gastric inhibitory peptide (GIP), glucagon-like peptide 1 (GLP-1), plasminogen activator inhibitor-1 (PAI-1). [§] Change means baseline minus final values. *n=22 and 21 individuals for the mango and placebo treatments, respectively. **n=24 and 22 individuals for the mango and placebo treatments, respectively.

Table 10. Effect of 90 day supplementation with mango or a placebo on plasma cytokines in individuals with moderately elevated blood glucose

Parameter	**Mango**			**Placebo**			**P value**			**P value**
	Baseline (n=21)	Final (n=21)	Change§	Baseline (n=20)	Final (n=20)	Change§	Time	Treatment	Time X Treatment	Change from baseline
IL-1β (*pg/mL*)	2.77± 3.6	2.4± 2.8	0.4± 2.0	2.78± 3.8	3.11 ± 3.6	-0.3± 1.8	0.9851	0.6343	0.6496	0.2422
IL-1ra (*pg/mL*)	89.31±101	85.75±78.3	3.6± 55.7	109.9± 109	113.59±121.2	-3.7± 52.7	0.9977	0.2917	0.8739	0.6705
IL-2 (*pg/mL*)	4.28±7.4	3.21±3.9	1.1± 5.9	5.65± 11.9	5.19±9.2	0.5± 6.1	0.6871	0.3792	0.8712	0.7448
IL-4 (*pg/mL*)	1.66±2.2	1.86±1.6	-0.2± 1.7	2.36±11.9	2.18±2.2	0.2± 1.8	0.9799	0.3111	0.7089	0.4945
IL-5 (*pg/mL*)	13.76±12.7	12.17±10.9	1.6± 9.2	13.05±11.1	13.89±12.5	-0.8± 8.7	0.8868	0.8468	0.6424	0.3901
IL-6 (*pg/mL*)	4±5.6	3.21±4.1	0.8± 3.6	4.97±6.5	5.16±7.2	-0.2± 3.4	0.8006	0.2753	0.6981	0.3522
IL-7 (*pg/mL*)	13.32±18.6	14.37±14.6	-1.0± 11.2	21.38±33.7	18.15±19.6	3.2± 16.8	0.8278	0.2407	0.6705	0.3415
IL-8 (*pg/mL*)	11.6±11	11.05±7.8	0.5± 7.2	15.75±15.9	13.92±12.8	1.8± 8.3	0.6603	0.1961	0.8127	0.6004
IL-9 (*pg/mL*)	24.53±19.2	23.82±14.1	0.7± 12.9	35.25±31.8	31.32±26.5	3.9± 10.7	0.6593	0.0860	0.7596	0.3895
IL-10 (*pg/mL*)	12.86±14.4	11.03±9.5	1.8± 9.0	19.99±26.8	16.94±17.5	3.1± 12.1	0.5423	0.1060	0.8790	0.7156
IL-12p70 (*pg/mL*)	31.48±44	20.33±20.2	11.2± 30.9	51.66±103.7	38.98±59.6	12.7± 47.7	0.4011	0.1730	0.9570	0.9032
IL-13 (*pg/mL*)	10.94±7.9	10.20±8.3	0.7± 5.2	21.36±28.1	16.45±15.7	4.9± 14.7	0.4511	**0.0284**	0.5777	0.2277
Eotaxin (*pg/mL*)	102.87±70.2	101.67±56	1.2± 38.7	102.07±53.4	111.73±53.5	-9.7± 34.2	0.7454	0.7227	0.6772	0.2775

Table 10. Cont.

Parameter	Mango			Placebo			P value			P value
	Baseline (n=21)	Final (n=21)	Change[§]	Baseline (n=20)	Final (n=20)	Change[§]	Time	Treatment	Time X Treat-ment	Change from baseline
Basic FGF (*pg/mL*)	48.11±47.8	39.95±33.5	8.2± 28.2	59.12±70	56.65±59.7	2.5± 27.6	0.6583	0.2507	0.8124	0.5163
GCSF (*pg/mL*)	71.68±54.3	68.7±38.9	3.0± 38.8	107.42±125.8	95±67.9	12.1± 68.4	0.6629	0.0778	0.7918	0.5986
GMCSF (*pg/mL*)	33.48±55.5	32.19±54	1.3± 12.4	27.81±51.4	21.47±36.6	6.3± 22.0	0.7310	0.4607	0.8198	0.3673
IFNγ (*pg/mL*)	102.7±114.3	118.11±95.1	-15.4± 87.1	135.47±119.6	139.82±111.6	-4.4± 85.9	0.6864	0.2675	0.8214	0.6851
IP-10 (*pg/mL*)	1165.82±655.6	1055.24±450.8	110.6± 462.0	1382.9±1076.4	1313.53±1089.5	69.4± 362.3	0.6458	0.2129	0.9136	0.7531
MCP-1 (*pg/mL*)	3.91±8.8	4±6.8	-0.1± 4.9	5.72±16.4	6.5±14	-0.8± 9.2	0.8701	0.4207	0.8977	0.7645
MIP-1α (*pg/mL*)	5.95±5.3	4.56±3.5	1.4± 3.1	7.55±9.7	6.66±7.2	0.9± 4.3	0.4499	0.2214	0.8679	0.6679
MIP-1β (*pg/mL*)	75.43±53.7	76.16±47.8	-0.7± 37.3	82.78±60.7	77.89±55.3	4.9± 21.4	0.8632	0.7071	0.8160	0.5600
PDGFBB (*pg/mL*)	138.19±184.73	159.12±184.2	-21.0± 212.0	137.42±115	184.26±202.1	-46.8± 191.8	0.3834	0.7535	0.7384	0.6842
RANTES (*pg/mL*)	4315.93±3348.6	5230.02±2987.4	-914.1+ 2865.2	5782.3±3246.5	4259.01±2582.8	1523+ 2406.8	0.6534	0.7150	0.0752	0.6842
TNF-α (*pg/mL*)	18.94±21.9	22.61±22.4	-3.7± 24.8	42.94±57.9	35.59±46.8	7.4± 19.7	0.8355	**0.0399**	0.5349	0.1240
VEGF (*pg/mL*)	14.06±20.5	10.32±13.7	3.7± 11.0	26.6±52.5	19.75±30.7	6.8± 25.0	0.4634	0.1301	0.8293	0.6066

Table 10. Cont.

Parameter	Mango			Placebo			P value			P value
	Baseline (n=21)	Final (n=21)	Change§	Baseline (n=20)	Final (n=20)	Change§	Time	Treatment	Time X Treatment	Change from baseline
TBARS (μM)*	28.9± 25.0	38.2± 75.6	-9.4± 83.2	40.1± 51.5	27.3± 16.2	12.7± 45.3	0.8895	0.9916	0.3665	0.3617
Anti-oxidants (mM)	3.0± 2.7	2.7± 2.1	0.3± 1.5	3.7± 7.6	4.2± 2.7	-0.5± 1.9	0.8446	**0.0371**	0.4049	0.1045

Values are mean ± standard deviation. Significance level set at *P* value <0.05. interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6(IL-6), interleukin- 7(IL-7), interleukin-8 (IL-8), interleukin- 9 (IL-9), interleukin-10 (IL-10), interleukin- 12p70(IL-12p70), interleukin-13 (IL-13), fibroblast growth factor (FGF), granulocyte colony-stimulating factor (GCSF), granulocyte macrophage colony-stimulating factor (GMCSF), interferon gamma (IFNγ), interferon gamma-induced protein -10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta(MIP-1β), platelet-derived growth factor-beta (PDGF-BB), chemokine ligand 5(RANTES), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF) thiobarbituric acid reactive substances (TBARS) *n=15 baseline and 15 final and 16 baseline and 16 final individuals for the mango and placebo treatments, respectively. § Change means baseline minus final values.

CHAPTER V

DISCUSSION AND CONCLUSION

This study investigated the effects of daily supplementation of freeze-dried mango (10 g/d) for 12 weeks on diabetes markers, anthropometrics, body composition, lipid parameters, and inflammatory markers in individuals with moderately elevated blood glucose. This study is a follow-up of our animal study that demonstrated that mango was able to modulate blood glucose in mice fed a high fat diet, a model of diet-induced obesity [19]. We have also conducted a clinical study that investigated the effects of daily supplementation of the same dose of freeze-dried mango on clinical parameters of obese individuals and found that mango supplementation improved blood glucose in these individuals after twelve weeks of supplementation [61]. The findings of the current study, however, showed that mango supplementation has no effect on glucose parameters in individuals with moderately elevated blood glucose. Because of the very sweet taste of the mango fruit, the popular belief is that consumption of this fruit is not a good choice for individuals at risk or with diabetes. However, our findings demonstrate that freeze-dried mango neither increases nor decreases blood glucose in these individuals.

We accept our null hypothesis that mango supplementation has no effect on fasting blood glucose in individuals with elevated blood glucose. Additionally, there was no significant

difference found in glycated hemoglobin A1C (HbA1C%), a measure of average plasma glucose concentration over a three month period. The National Institute of Health categorized HbA1C% values less than 5.7% to be normal while values between 5.7% and 6.4% are a diagnosis for pre-diabetes [63]. The average HbA1C in our study was 5.4% for both groups, which falls in the category for normal healthy individuals. Mango supplementation may not have had an effect on blood glucose and HbA1C% in our study participants because it was not high enough to correct. Nonetheless, mango supplementation did not cause negative effects on blood glucose nor HbA1C. Our screening for study participants only used a finger stick fasting blood glucose assessment using a home testing glucometer and not based on HbA1C. If there are no budgetary or other experimental constraints, future studies should use HbA1C as the screening tool to identify study participants.

No changes in fasting blood glucose, insulin, or HOMA-IR were observed after 12 weeks of mango supplementation. We also did not see changes in glucose tolerance testing measures in either treatment group. Our findings are not in agreement with the findings of Evans *et al*. where they saw a significant difference in fasting blood glucose with mango supplementation in obese individuals [61]. Differences in these findings could be due to different effects in obese individuals or adherence to the study protocol of consuming the supplement daily. Perhaps mango has an effect on glucose uptake in the adipose tissue such that a glucose lowering effect was observed by Evans and colleagues [61]. The effect of mango supplementation on adipose tissue has also been demonstrated by Lucas and colleagues [19]. They found that 1% mango supplementation for two months was able to significantly decrease body fat in rats fed a high fat diet [19]. However, when a similar dose of mango was consumed by obese individuals there were no significant differences found in fat mass after 12 weeks of supplementation but a reduction in fasting blood glucose was observed [61]. To examine whether the effects of mango on blood glucose is dependent on

adiposity, we separated and analyzed the data for the obese (BMI > 30 kg/m^2) participants in the current study. Mango was still not able to lower blood glucose in obese individuals.

Very few studies have examined the glucose-lowering effect of the mango fruit. Most of the studies investigating the glucose-lowering effect of mango have used an extract of the different parts of the mango plant. Aderibigbe and colleagues found that aqueous extract of mango leaves modulated fasting blood glucose level and glucose tolerance test in rats with glucose-induced hyperglycemia [55]. The aqueous mango leaf extract was given orally to one group 60 minutes prior to the glucose challenge and in another group the extract was given together with the glucose challenge. Both groups had lower blood glucose levels at 30 and 60 minutes; however, rats given the extract prior to the glucose challenge maintained blood glucose level up to 120 minutes [55]. These findings could indicate that a higher concentration of the bioactive compound in mango (i.e. extract) may be needed for glucose lowering effects. This could also indicate that the timing of mango supplementation may play a role in its glucose-lowering capabilities. Since the mango extract given 60 minutes prior to the glucose challenge resulted to a lower blood glucose level concentration for a longer period, perhaps mango needs to be consumed before a meal to induce a more profound glucose lowering properties. It has been suggested that the fiber content of the mango slows down gut transit time and therefore slows down the absorption of glucose [58]. It is possible that we might need to have our study participants consume the freeze-dried mango supplements at strategic times before meals and not just once a day as our protocol required. However, this is a speculation and needs to be further investigated.

The component of the mango fruit responsible for lowering blood glucose is not clear. Previous studies have attributed the glucose lowering effect of mango to be due to its fiber content [58]. A meta-analysis found that an increase in fiber intake by approximately 18 g/d can reduce fasting blood glucose and HbA1C [64]. The American Dietetic Association recommends 30-50 g/d dietary fiber for individuals with T2D [64]. The mean total fiber intake of our study

participants for both groups (16-18 g fiber/d without the mango supplementation which provided 1.29 g/d) is much lower than the recommended fiber intake and lower in studies that found a correlation between fiber intake and blood glucose. Perhaps, a larger dose of mango would be needed to provide the amount of fiber these studies found to be beneficial.

The glucose-lowering properties of mango have been compared to other fruits. Roongpisuthipong and colleagues measured post-prandial glucose and insulin response in diabetic female participants. They found that when compared to banana, durian, rambutan, and pineapple, mango had a significantly lower post-prandial plasma glucose and insulin response [59]. Edo and colleagues found that mango had one of the lowest mean postprandial glucose levels and the lowest peak postprandial glucose level when compared to banana, orange, pineapple, and pawpaw [58]. This shows that even though we did not see a glucose lowering effect with chronic mango supplementation, it might have an effect on post-prandial response.

In this study, we did not observe differences in anthropometrics parameters among the treatment groups. However, we did not expect to see differences in anthropometrics parameters since the subjects were asked to maintain their current diet and physical activity patterns. The study participants had normal blood pressure throughout the study and no changes in waist or hip circumference were observed. Both groups had an average BMI of 31 kg/m^2, which is classified as obese. Obesity is a risk factor for T2D and mango supplementation did not have any effects on BMI. Additionally, no differences were found in body composition between the treatment group and the control. This is consistent with the findings of Evans *et al.,* which did not see significant changes in body weight and composition after mango supplementation [61].

We saw minimal significant changes in the lipid profile of subjects on either treatment. Saleh and colleagues found that 20 mg/kg/d mangiferin supplementation in high fat fed streptozotocin (STZ)-induced diabetic rats reduced circulating and liver triglycerides and

cholesterol [57]. Our findings were also not consistent with the findings of Robles-Sanchez and colleagues which found both whole mango and fresh cut mango to significantly lower fasting plasma triglycerides and VLDL after 30 days of mango supplementation in subjects considered to be in good health [60]. Each subject was asked to consume 200 g mango per day. Our study participants consumed 10 g freeze-dried mango per day which is equivalent to about 50-100 g fresh mango or half a fruit. Perhaps a higher dose of freeze-dried mango or fresh mango is needed to affect blood lipids. Another study by Muruganandan and colleagues also showed that mango supplementation improved lipid profiles in rats. Mango extract (10 and 20 mg/kg i.p.) was as effective as insulin (6 U/kg i.p.) at lowering blood glucose, triglycerides, total cholesterol, LDL cholesterol, and increasing HDL cholesterol after just 28 days of supplementation in STZ-induced diabetic rats [14]. These two studies show that mango is capable of improving blood lipids in diabetic rats and also in humans when the mango supplement is given in large enough amounts.

One finding in this study was a significant increase in bilirubin in both groups after 90 days of supplementation. An animal study done by Fu and colleagues looked at two strains of rat (Winstar and Gunn rats) and bilirubin levels. The Gunn rat is used as a model for jaundice because they lack the enzyme bilirubin glucuronide preventing bilirubin to be combined with bile and thus accumulating in the blood. They found that in the Gunn rats, the excess bilirubin was able to stabilize plasma insulin levels of the STZ-induced diabetic rats but not in Winstar rats [65]. This may shows that bilirubin has the ability to preserve insulin secretion from the pancreatic islets in hyperbilirubinemic animals. They found that bilirubin concentrations as low as 1.7 gm/dL prevented apoptosis in rat insulinoma cells [65]. The National Health and Nutrition Examination Survey (NHANES) also reported that higher bilirubin levels may protect against T2D [66, 67]. Our study participants in both groups experienced an average increase of 0.1 gm/dL bilirubin after 90 days of supplementation. While still within the normal range, the mean bilirubin values are still much lower than the values seen in previous studies [65, 67]. A longer period of

supplementation may be required to induce a greater increase in bilirubin in individuals with elevated blood glucose.

Of the diabetes parameters measured, C-peptide, GIP, and GLP-1, all markers of insulin production, did not change before and after the twelve-week supplementation with mango or placebo. Visfatin, an enzyme that promotes β-cell maturation and is therefore associated with insulin production did not change throughout the study. This supports our findings of mango not having an effect on blood glucose since insulin allows for the entry of glucose into the cell. Evans and colleagues saw a mild increase in insulin of male obese subjects after twelve weeks of mango supplementation but not in obese female subjects [61]. A study conducted by Sellamuthu and colleagues found that mangiferin protected pancreatic β-cells from oxidative stress and therefore increased insulin secretion and decreased blood glucose in STZ-induced diabetic rats [68]. The discrepancies in the findings of Sellamuthu and colleagues and that of Evans *et al*. and our present study could be due to the dose of mango used and possibly the amount of phenolic compound mangiferin [61, 68].

Ghrelin, leptin and adiponectin, all involved in metabolic processes, were not changed with mango supplementation. Saleh and colleagues found that 20 mg/kg/d mangiferin supplementation increased adiponectin in STZ-induced diabetic rats after 28 days of supplementation [57]. Our findings show that mango does not have an effect on these parameters in individuals with moderately elevated blood glucose. Glucagon, which raises the concentration of glucose in the blood, was also not affected by freeze-dried mango supplementation. PAI-1 and resistin, both correlated with increased adiposity did not differ from baseline to final. There was also no difference in body composition or percent body fat so we would not expect for PAI-1 or resistin levels to change. Mango did not differ from the control group in any of these parameters suggesting that mango did not increase the risk of T2D.

Chronically elevated inflammatory cytokines are typically present in disease states like T2D [69]. The increase in inflammatory cytokines can cause damage by producing an excess amount of reactive oxygen species (ROS). A surplus of ROS can result in oxidative stress causing damage to proteins, lipids, and DNA as well as disrupting cellular signaling, contributing to the damage and dysfunction [35]. Of the twenty-five cytokines measured, we did not see any significant changes. A previous human study found that the concentrations of the inflammatory cytokines TNF-α and MCP-1, increased in the presence of diabetes [69]. Moreover, Saleh and colleagues found that mangiferin (20 mg/kg/d) supplementation for 28 days decreased serum TNF-α in high-fat diet fed STZ-induced diabetic rats [57]. Mangiferin was also able to cause a significant reduction in airway inflammation and a positive immunomodulatory effect (increased IL-2, IL-10, IL-12, IFN-γ and decreased IL-3, IL-4, IL-5, IL-9, IL-13, IL-17, RANTES, and TNF) in a mouse model of chronic respiratory disease [70]. Perhaps we did not see any differences in some of these cytokines because they are not directly associated with diabetes.

Thiobarbituric acid reactive substances (TBARS), which assess lipid peroxidation was not affected by mango supplementation. Rodriguez and colleagues incubated red blood cells with H_2O_2 and mangiferin and measured lipid peroxidation by TBARS. Mangiferin (1µg/mL) decreased lipid peroxidation and a higher dose (100 µg/mL) decreased the damage to that of the cells not treated with H2O2 [71]. Sellamuthu and colleagues found that in STZ- induced diabetic rats, mangiferin (40 mg/kg/d) supplementation decreased TBARS after 30 days of treatment [68]. The discrepancy with the in vitro and animal studies and our findings of no effects on TBARS concentrations may be due to low bioavailable concentrations of mangiferin from our freeze-dried mango.

We observed significantly lower plasma total antioxidants in the mango group compared to the control group. These results were very surprising considering ¾ cup of mango contains 27.7 mg of vitamin C and 4.2 mg of vitamin E, while the placebo does not contain any

antioxidants. The food recalls collected three times during each arm of the study showed no difference in the dietary anti-oxidant between the mango and placebo groups. It is uncertain why the control group has higher plasma antioxidant but this may help explain the lack of significance in most of the parameters measured. A meta-analysis of 14 studies showed that antioxidant supplementation does not affect plasma glucose and insulin levels, yet antioxidant supplementation significantly reduced HbA1C level [72]. Curiously, Savu and colleagues found that total antioxidant activity as well as residual antioxidant activity was significantly higher in patients with uncomplicated T2D compared to a healthy control [73]. It is known that antioxidants improve oxidative stress such as those generated by complications of diabetes but some contradicting results are also available.

There were no significant differences for either group considering food intake or physical activity, except that vitamin C intake tended to increase for the mango group from baseline to final. The study participants were asked to not make any changes to their dietary and physical activity habits during their participation in the study. The tendency of vitamin C to increase in the mango group during the study could be due to the study participants consuming the supplement by adding it to a cup of orange juice.

This study is not without limitations. For one, we cannot be certain whether the study participants stored the supplements properly or consumed the supplements daily. Although they were given calendars to record their intake of the supplements, over-reporting can still occur. It would have been better if we can observe the participants actually consuming the supplements or if we can assess some markers associated with mango consumption such as mangiferin levels. A small sample size may allow for the presence of outliers to have a greater influence on the results. Using a twelve week intervention period may not have been enough time for the freeze-dried mango supplement to cause significant changes in individuals with moderately elevated blood glucose. Another limitation of this study is that a home glucometer testing kit was used for the

screening of the study participants. The home glucometer measures whole blood glucose which makes the screening blood glucose on the high side. When plasma was used, the average fasting blood glucose as well as HbA1C is within normal range. This limited our study because our subjects did not have a blood glucose value significantly high enough to correct. Another limitation is the dose of mango used in the study. The study subjects were asked to consume 10 g/d mango, which is much lower than some previous studies [14, 60, 68]. Though 10 g/d is a practical amount of supplement to consume, it may not be enough to induce positive effects.

Although we did not see a reduction in blood glucose parameters with twelve weeks of freeze-dried mango supplementation, our findings indicate that chronic consumption of mango by individuals with moderately elevated blood glucose does not cause negative changes in blood glucose, body weight, lipid parameters, and inflammatory markers associated with T2D. Our findings also indicate that mango may have protective effects by increasing bilirubin levels. Additional clinical trials with longer duration of mango supplementation and larger sample sizes still need to be conducted.

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VITA

Jessica Michelle Semkoff

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF MANGO SUPPLEMENTATION ON CLINICAL PARAMETERS OF INDIVIDUALS WITH MODERATELY ELEVATED BLOOD GLUCOSE

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in May, 2015.

Completed the requirements for the Bachelor of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in May, 2013.

Experience:

Graduate Research Assistance Fall 2014- Spring 2015

Professional Memberships:

Academy of Nutrition and Dietetics (AND)