Effect of Naringin on Insulin Resistance and Oxidative Stress in Fructose Fed Rats

Wachirawadee Malakul* and Sirinat Pengnet

Department of Physiology, Faculty of Medical Science, Naresuan University, Phisanulok 65000

* Corresponding author. E-mail address: wachirawadeem@hotmail.com

Received: 19 May 2017; Accepted: 21 July 2017

Abstract

Consumption of fructose is associated with the development of insulin resistance. Naringin, the major grapefruit flavonoid, has antioxidant, lipid lowering, insulin sensitizing, and cardiovascular protective activities. Therefore, the aim of this study was to evaluate the effect of naringin treatment on insulin resistance and oxidative stress in fructose fed rats. Male rats were divided into three groups: control (C), fructose (F), and fructose+ naringin (FN). Fructose fed rats received 10% fructose (w/v) in the drinking water for 12 weeks. Naringin (100 mg/kg/day) was orally administered for the final 4 weeks of the study. At the end of the study, the levels of insulin and blood glucose, as well as an insulin resistance index (HOMA-IR) were determined. Hepatic and serum levels of malondialdehyde (MDA) and antioxidant enzyme, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured. Results showed that fructose-fed rats exhibited a significant increase in serum insulin, glucose, and HOMA-IR index. Fructose feeding also increased hepatic and serum MDA levels. Treatment of the fructose-fed rats with nargin reversed these alterations. These results suggest that naringin treatment for 4 weeks improves high fructose induced insulin resistance and oxidative stress in rats.

Keywords: Naringin, Fructose, Insulin resistance, Oxidative stress

Introduction

The use of fructose in soft drinks and food products is dramatically increased in recent decades (Tappy, Le, Tran, & Paquot, 2010). It is now widely recognized that both sedentary lifestyle and unhealthy dietary habits contribute to the development of metabolic disorders (Stanhope, 2016). There is increasing evidence that the consumption of high amounts of fructose, mainly through soft drinks, increases the risk of metabolic disturbance including hyperglycemia, insulin resistance, and hyperinsulinemia (Ferder, Ferder, & Inserra, 2010; Ter Horst, Schene, Holman, Romijn, & Serlie, 2016). Insulin resistance is a pathological condition in which tissues cannot fully respond to the hormone insulin. This hormone regulates the blood glucose levels in body by promoting glucose uptake into body cells and regulating carbohydrate and protein metabolism Insulin resistance plays an important role in the initiation of type 2 diabetes, a major risk factor for cardiovascular disease (Tangvarasittichai, 2015; Tran, Yuen, & McNeill, 2009).

There is evidence demonstrated that oxidative stress is closely associated with insulin resistance (Rains & Jain, 2011; Tangvarasittichai, 2015). An elevation of reactive oxygen species (ROS) generation results in damage to lipid, protein, and other molecules, which may contribute to the development of insulin resistance (Rains & Jain, 2011; Tangvarasittichai, 2015). Fructose consumption has also been reported to increase oxidative stress and induce insulin resistance (Hininger-Favier, Benaraba, Coves, Anderson, & Roussel, 2009; Murunga et al., 2016). There are several studies demonstrating that administration of antioxidant agents to rodents can decrease plasma insulin, glucose, and ameliorate insulin resistance in fructose fed conditions (Faure et al., 1997; Hininger-Favier et al., 2009; Suwannaphet, Meeprom, Yibchok-Anun, & Adisakwattana, 2010).

Therefore, a decrease in ROS formation might be a therapeutic approach to reduce fructose induced insulin resistance.

Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside), the major compound of flavonoid found in grapefruit, is reported to possess beneficial pharmacological effects, including antihyperlipidemic, antioxidant, and antihyperglycemic activities in vivo models (Adebiyi, Adebiyi, & Owira, 2016; Alam, Kauter, & Brown, 2013; Mahmoud, Ashour, Abdel-Moneim, & Ahmed, 2012; Pu et al., 2012). In animal experiments, naringin could also improve insulin signaling (Alam et al., 2013). However, no studies were undertaken on the effect of naringin against high fructose-induced insulin resistance and oxidative stress. Therefore, the aim of present study was to investigate whether naringin treatment reduces insulin resistance and oxidative stress in fructose fed rats.

Methods and Materials

Chemicals

Naringin, fructose, and carboxymethylcellulose (CMC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rat/Mouse Insulin ELISA kit was obtained from Merck Milipore (Madrid, Spain). Naringin was dissolved in 0.1%CMC before use.

Animals and experimental protocol

Male Sprague-Dawley (SD) rats weighing 180-200 g were obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. All animals used in this study were housed at a constant temperature of 20 - 22°C, with a 12-h light-dark cycle and were allowed free access to tap water and the standard rat chow for one week. After acclimatization, rats were randomly divided into the following groups (8 rats per group): control (C), receiving tap water ad libitum; fructose (F), receiving fructose solution and vehicle 0.1% CMC; and fructose+naringin (FN), receiving fructose solution and naringin (100 mg/kg body weight/d). Fructose solution was administered to the rats as 10% fructose (w/v) in drinking water *ad libitum* for 12 weeks. Naringin (100 mg/kg body weight /d) was dissolved in 0.1% CMC and orally administrated to rats during the last four weeks of fructose feeding. The consumed fructose solution were measured daily. All animal procedures were performed within the institutional guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Naresuan University, Thailand.

Sample preparation

At the end of 12 weeks of fructose feeding, overnight fasted rats were euthanized with intraperitoneal pentobarbital (50 mg/kg). Blood samples were collected by cardiac puncture and subsequently centrifuged at 10,000*g* for 15 min at 4 °C. Serum supernatants were subsequently separated for biochemical analysis. Liver tissue of animals was rapidly dissected and immediately homogenized in ice cold phosphate buffer (50 mm, pH 7.4). The homogenate was then centrifuged at 3000 g for 20 min at 4 °C and the supernatant was collected for subsequent measurement of MDA, SOD, and GPx.

Measurement of glucose, insulin and advanced glycation end products (AGEs)

Fasting blood glucose levels were measured using a glucometer (Accu-Chek, Roche Diagnostic, France). The total insulin concentration in the serum was measured by using Rat/Mouse Insulin ELISA kit (Merck Milipore, Madrid, Spain) according to manufacturer's instructions. Insulin resistance was determined using the homeostasis model assessment index for insulin resistance (HOMA-IR) formula: HOMA-IR = [fasting insulin (ng/ml) x fasting glucose (mg/dL)] /405 (Chutia & Lynrah, 2015).

The serum AGE formation was determined according to a previous method (El-Bassossy, Badawy, Neamatallah, & Fahmy, 2016). Briefly, serum was diluted in saline (1:15 v/v) and the fluorescence intensity was measured at an excitation wavelength of 370 nm and emission wavelength of 440 nm.

Determination of oxidative stress.

Level of malondialdehyde (MDA) was determined using the TBRAS assay kit (Cayman Chemical, Ann Arbor, MI, USA). Briefly, supernatants from liver homogenates or serum (0.1 mL) was mixed with a sodium dodecyl sulfate solution (0.1 mL) and color reagent (4 mL). The reaction mixture was heated in a boiling water bath at 95°C for 60 minutes and then rapidly cooled. After centrifugation, the absorbance of the supernatant was measured at 532 nm.

The activity of superoxide dismutase (SOD) in the liver homogenates and serum was determined using the superoxide dismutase kit (Cayman Chemical, Ann Arbor, MI, USA) in accordance with the manufacturer's instructions. Briefly, supernatants from liver homogenates or serum was suspended in radical detector. The reaction was started by addition of xanthine oxidase. After an incubation of 30 minutes at room temperature, the absorbance was measured at 450 nm.

The glutathione peroxidase (GPx) activity was determined using a GPx assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. Briefly, supernatants from liver homogenates or serum (20 µL) was mixed with 100 µL of assay buffer (50 mM Tris-HCl, pH 7.6, containing 5 mM EDTA) and 50 µL of co-substrate mixture (lyophilized powder of NADPH, glutathione, and glutathione reductase). The reaction was initiated by addition of cumene hydroperoxide (20 µL). Absorbance at 340nm was measured at 60 sec intervals for 6 min.

Statistics

Results are expressed as the mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism 4.0 software, San Diego, USA). $P < 0.05$ was considered statistically significant.

Results

Effect of naringin on blood glucose, insulin and advanced glycation end products (AGEs)

There was no difference in fluid intake among the three experimental groups (control 43± 6; fructose 37±8; fructose+naringin 38±7 mL/day/rat). Rats treated with 10% (w/v) fructose in drinking water significantly increased levels of blood glucose and serum insulin, and HOMA-IR index when compared to control group. Treatment with naringin for 4 weeks reversed these parameters in fructose fed rats (Figure 1). Serum AGEs formation of the F group significantly increased in comparison with that of the C group. However, treatment of fructose fed rats with naringin decreased fructose induced AGE formation (Table 1).

Effect of naringin on oxidative stress

In fructose fed rats, the serum and liver MDA levels, a biomarker of lipid peroxidation, were statistically higher than those of control rats. However, activities of antioxidant SOD and GPx in both serum and liver of fructose fed rats were not significantly different from those of control rats. Oral administration of naringin to fructose fed rats caused a significant reduction in MDA levels, but had no effect on antioxidant activities in both serum and liver (Table 1 &2)

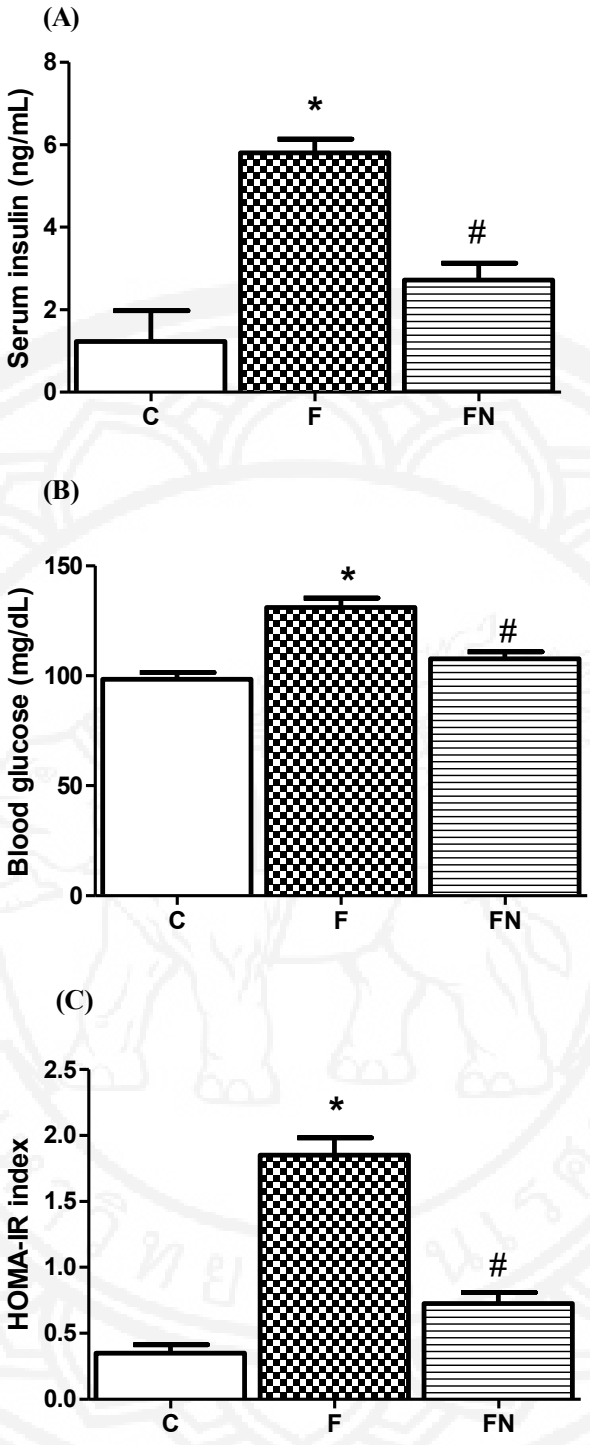

Figure 1 Effect of naringin treatment on the levels of blood glucose (A), serum insulin (B), and HOMA-IR index (C) in fructose fed rats. Data are expressed as mean ± SEM (n=8 in each group). Control(C), fructose fed rats (F), fructose fed rats treated with naringin (100 mg/kg/day) (FN).*P < 0.05 as compared to control; #P < 0.05 as compared to fructose.

Table 1 Effect of daily oral administration of naringinon on levels of serum MDA, SOD, GPx, and AGEs in fructose-fed rats

Group	MDA (μM/mL)	SOD (U/mL)	GPx (μU/mL)	AGE (fluorescent units)
C	0.8 ± 0.06	30.3 ± 0.9	90± 7.8	51±5
F	1.3 ± 0.06*	28.9 ± 1.9	80± 5.4	79±5*
FN	0.7 ± 0.07#	33.7 ± 4.6	93± 8.9	49±3#

Values are expressed as the mean ± SEM; *P < 0.05compared with control group; #P < 0.05 compared with F group.

Table 2 Effect of daily oral administration of naringinon on levels of hepatic MDA, SOD, and GPx in fructose-fed rats

Group	MDA (nM/mg protein)	SOD (U/mg protein)	GPx (μU/mg protein)
C	10.5± 1.1	60.3 ±4.9	58± 6.7
F	19.1±2.6*	52.8±5.8	45± 5.9
FN	12.3± 1.7#	65.9± 6.6	56± 6.1

Values are expressed as the mean ± SEM; *P < 0.05compared with control group; #P < 0.05 compared with F group.

Discussion

Chronic consumption of dietary sugars is associated with accelerating insulin resistance which precedes the development of type 2 diabetes (Shanik et al., 2008). Insulin resistance refers to the condition of reduced cellular sensitivity to hormone insulin, which mediates glucose uptake. This leads to decreased glucose metabolism and increased blood glucose concentration. The decreased responsiveness of tissues to insulin leads to the pancreas compensation and subsequently increased insulin secretion to maintain blood glucose concentration (Shanik et al., 2008). Fructose is widely used as a sweetener in several foods and beverages (Basaranoglu, Basaranoglu, Sabuncu, & Senturk, 2013; Hanover & White, 1993). Numerous studies have been shown that high fructose consumption caused hyperinsulinemia and insulin resistance in animals (Hozayen, Mahmoud, Soliman, & Mostafa, 2016; Putakala, Gujjala, Nukala, & Desireddy, 2017). The insulin resistance is commonly determined by the homeostatic model assessment of insulin resistance (HOMA-IR) index, which calculated by both fasting blood glucose and insulin levels (Tangvarasittichai, 2015; Wallace & Matthews, 2002).

Our results showed that the administration of fructose (10% w/v) in drinking water to rats for 12 weeks causes the development of insulin resistance as indicated by the significant increase in blood glucose and serum insulin levels and HOMA-IR values. This is in agreement with previous studies reporting the development of insulin resistance in fructose fed animals (El-Bassossy et al., 2016; Hininger-Favier et al., 2009). However, the mechanisms of insulin resistance due to fructose intake are not completely understood. Several hypotheses have been proposed to explain the development of insulin resistance in fructose fed conditions, including an impairment of intracellular insulin signaling pathway, a change in the activities of several enzymes regulating hepatic glucose metabolism, and a generation of reactive oxygen species (ROS) (Basciano, Federico, & Adeli, 2005; Tran et al., 2009).

The present study demonstrated that naringin treatment for 4 weeks lowered blood glucose and insulin, and improved insulin resistance in fructose fed rats. It has been demonstrated that naringin reduced the levels of blood glucose and insulin in high fat fed mice and diabetic rats (Mahmoud et al., 2012). In type 2 diabetic *db/db* mice, naringin increased glycolysis and decreased gluconeogenesis in liver, leading to reduced blood glucose levels (Jung,

Lee, Jeong, & Choi, 2004). In mice fed high fat diet, naringin could improve insulin resistance via activation of the AMP activated protein kinase pathway (Pu et al., 2012). Therefore, naringin might have a beneficial effect against the development of insulin resistance induced by high fructose intake.

In addition to insulin resistance, high fructose consumption could also trigger ROS generation. After absorption, fructose is primarily metabolized via fructolysis in the liver. Rapid fructolysis results in ATP depletion, resulting in enhancing oxidative stress, which causes the cellular function disruption and damage (Zhang, Jiao, & Kong, 2017). Furthermore, there is an evidence that fructose and its metabolites cause advanced glycation end products (AGEs) formation and subsequently ROS production, leading to lipid peroxidation (Zhang et al., 2017). MDA levels have been used as the indicator of oxidative damage initiated by ROS. Oxidative stress has been shown to play an important role in the development and progression of insulin resistance (Rains & Jain, 2011; Tangvarasittichai, 2015). It has been reported that insulin signaling was impaired under oxidative stress conditions, leading to insulin resistance of the cell (Rains & Jain, 2011). In animal experiments, overproduction of ROS in liver and adipose tissue induced the development of insulin resistance (Matsuzawa-Nagata et al., 2008). Our results were consistent with previous studies demonstrating that fructose feeding elevated oxidative stress as evidenced by an increase in serum MDA levels (Develi-Is et al., 2014; Mahmoud & Elshazly, 2014). In addition, our study also found that there were no changes in antioxidant enzyme activities (SOD and GPx) in serum and liver homogenates among the three experimental groups. However, previous studies have reported increase in (Demirtas, Pasaoglu, Bircan, Kantar, & Turkozkan, 2015) and decrease in (Ajiboye et al., 2016; Dornas et al., 2013) antioxidant enzyme activities in fructose fed animals. One possible explanation for a lack of significant change in the activity of SOD and GPx in the present study probably due to the different concentrations of sugar used or a degree of severity of metabolic abnormalities. Furthermore, serum AGEs levels were also elevated in fructose fed rats. Naringin has been reported to have free radical scavenging and antioxidant properties in several animal models (Mahmoud et al., 2012; Murunga et al., 2016). In our study, administration of naringin decreased serum TBARS and AGEs levels in fructose fed rats. These results suggested that naringin could inhibit high fructose induced oxidative stress damage in rats.

Conclusion and Suggestion

The present study demonstrates that high fructose intake induces insulin resistance and oxidative stress in rats. Oral administration of naringin for 4 weeks ameliorates high fructose induced insulin resistance and oxidative stress. Our findings provide the beneficial effect of naringin for treating fructose induced insulin resistance. However, further studies are needed to explain the exact underlying mechanisms of insulin sensitizing effect of naringin in fructose fed conditions.

Acknowledgements

This research was supported by grants from the National Research Council of Thailand.

References

Adebiyi, O. A., Adebiyi, O. O., & Owira, P. M. (2016). Naringin Reduces Hyperglycemia-Induced

Cardiac Fibrosis by Relieving Oxidative Stress. *PLoS One, 11*(3), e0149890. doi:10.1371/journal.pone.0149890

Ajiboye, T. O., Raji, H. O., Adeleye, A. O., Adigun, N. S., Giwa, O. B., Ojewuyi, O. B., & Oladiji, A. T. (2016). Hibiscus sabdariffa calyx palliates insulin resistance, hyperglycemia, dyslipidemia and oxidative rout in fructose-induced metabolic syndrome rats. *J Sci Food Agric, 96*(5), 1522-1531. doi:10.1002/jsfa.7254

Alam, M. A., Kauter, K., & Brown, L. (2013). Naringin improves diet-induced cardiovascular dysfunction and obesity in high carbohydrate, high fat diet-fed rats. *Nutrients, 5*(3), 637-650. doi: 10.3390/nu5030637

Basaranoglu, M., Basaranoglu, G., Sabuncu, T., & Senturk, H. (2013). Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol, 19*(8), 1166-1172. doi:10.3748/wjg.v19.i8.1166

Basciano, H., Federico, L., & Adeli, K. (2005). Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond), 2*(1), 5. doi: 10.1186/1743-7075-2-5

Chutia, H., & Lynrah, K. G. (2015). Association of Serum Magnesium Deficiency with Insulin Resistance in Type 2 Diabetes Mellitus. *J Lab Physicians, 7*(2), 75-78. doi:10.4103/0974-2727.163131

Demirtas, C. Y., Pasaoglu, O. T., Bircan, F. S., Kantar, S., & Turkozkan, N. (2015). The investigation of melatonin effect on liver antioxidant and oxidant levels in fructose-mediated metabolic syndrome model. *Eur Rev Med Pharmacol Sci, 19*(10), 1915-1921.

Develi-Is, S., Ozen, G., Bekpinar, S., Topal, G., Unlucerci, Y., Dogan, B. S., & Uysal, M. (2014). Resveratrol improves high-fructose-induced vascular dysfunction in rats. *Can J Physiol Pharmacol, 92*(12), 1021-1027. doi:10.1139/cjpp-2014-0245

Dornas, W. C., de Lima, W. G., dos Santos, R. C., Guerra, J. F., de Souza, M. O., Silva, M., . . . Silva, M. E. (2013). High dietary salt decreases antioxidant defenses in the liver of fructose-fed insulin-resistant rats. *J Nutr Biochem, 24*(12), 2016-2022. doi:10.1016/j.jnutbio.2013.06.006

El-Bassossy, H., Badawy, D., Neamatallah, T., & Fahmy, A. (2016). Ferulic acid, a natural polyphenol, alleviates insulin resistance and hypertension in fructose fed rats: Effect on endothelial-dependent relaxation. *Chem Biol Interact, 254*, 191-197. doi:10.1016/j.cbi.2016.06.013

Faure, P., Rossini, E., Lafond, J. L., Richard, M. J., Favier, A., & Halimi, S. (1997). Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. *J Nutr, 127*(1), 103-107.

Ferder, L., Ferder, M. D., & Inserra, F. (2010). The role of high-fructose corn syrup in metabolic syndrome and hypertension. *Curr Hypertens Rep, 12*(2), 105-112. doi:10.1007/s11906-010-0097-3

Hanover, L. M., & White, J. S. (1993). Manufacturing, composition, and applications of fructose. *Am J Clin Nutr, 58*(5 Suppl), 724s-732s.

Hininger-Favier, I., Benaraba, R., Coves, S., Anderson, R. A., & Roussel, A. M. (2009). Green tea extract decreases oxidative stress and improves

insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. *J Am Coll Nutr, 28*(4), 355-361.

Hozayen, W. G., Mahmoud, A. M., Soliman, H. A., & Mostafa, S. R. (2016). Spirulina versicolor improves insulin sensitivity and attenuates hyperglycemia-mediated oxidative stress in fructose-fed rats. *J Intercult Ethnopharmacol, 5*(1), 57-64. doi:10.5455/jice.20151230055930

Jung, U. J., Lee, M. K., Jeong, K. S., & Choi, M. S. (2004). The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. *J Nutr, 134*(10), 2499-2503.

Mahmoud, A. A., & Elshazly, S. M. (2014). Ursodeoxycholic acid ameliorates fructose-induced metabolic syndrome in rats. *PLoS One, 9*(9), e106993. doi:10.1371/journal.pone.0106993

Mahmoud, A. M., Ashour, M. B., Abdel-Moneim, A., & Ahmed, O. M. (2012). Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats. *J Diabetes Complications, 26*(6), 483-490. doi:10.1016/j.jdiacomp.2012.06.001

Matsuzawa-Nagata, N., Takamura, T., Ando, H., Nakamura, S., Kurita, S., Misu, H., . . . Kaneko, S. (2008). Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. *Metabolism, 57*(8), 1071-1077. doi: 10.1016/j.metabol.2008.03.010

Murunga, A. N., Miruka, D. O., Driver, C., Nkomo, F. S., Cobongela, S. Z., & Owira, P. M. (2016). Grapefruit Derived Flavonoid Naringin Improves Ketoacidosis and Lipid Peroxidation in Type 1 Diabetes Rat Model. *PLoS One, 11*(4), e0153241. doi:10.1371/journal.pone.0153241

Pu, P., Gao, D. M., Mohamed, S., Chen, J., Zhang, J., Zhou, X. Y., . . . Jiang, H. (2012). Naringin ameliorates metabolic syndrome by activating AMP-activated protein kinase in mice fed a high-fat diet. *Arch Biochem Biophys, 518*(1), 61-70. doi: 10.1016/j.abb.2011.11.026

Putakala, M., Gujjala, S., Nukala, S., & Desireddy, S. (2017). Beneficial Effects of Phyllanthus amarus Against High Fructose Diet Induced Insulin Resistance and Hepatic Oxidative Stress in Male Wistar Rats. *Appl Biochem Biotechnol*. doi: 10.1007/s12010-017-2461-0

Rains, J. L., & Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radic Biol Med, 50*(5), 567-575. doi:10.1016/j.freeradbiomed.2010.12.006

Shanik, M. H., Xu, Y., Skrha, J., Dankner, R., Zick, Y., & Roth, J. (2008). Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care, 31 Suppl 2*, S262-268. doi: 10.2337/dc08-s264

Stanhope, K. L. (2016). Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit Rev Clin Lab Sci, 53*(1), 52-67. doi:10.3109/10408363.2015.1084990

Suwannaphet, W., Meeprom, A., Yibchok-Anun, S., & Adisakwattana, S. (2010). Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. *Food Chem Toxicol, 48*(7), 1853-1857. doi:10.1016/j.fct.2010.04.021

Tangvarasittichai, S. (2015). Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes*, *6*(3), 456-480. doi: 10.4239/wjd.v6.i3.456

Tappy, L., Le, K. A., Tran, C., & Paquot, N. (2010). Fructose and metabolic diseases: new findings, new questions. *Nutrition*, *26*(11-12), 1044-1049. doi:10.1016/j.nut.2010.02.014

Ter Horst, K. W., Schene, M. R., Holman, R., Romijn, J. A., & Serlie, M. J. (2016). Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: a systematic review and meta-analysis of diet-intervention trials. *Am J Clin Nutr*, *104*(6), 1562-1576. doi:10.3945/ajcn.116.137786

Tran, L. T., Yuen, V. G., & McNeill, J. H. (2009). The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. *Mol Cell Biochem*, *332*(1-2), 145-159. doi: 10.1007/s11010-009-0184-4

Wallace, T. M., & Matthews, D. R. (2002). The assessment of insulin resistance in man. *Diabet Med*, *19*(7), 527-534.

Zhang, D. M., Jiao, R. Q., & Kong, L. D. (2017). High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions. *Nutrients*, *9*(4). doi: 10.3390/nu9040335