AN ABSTRACT OF THE THESIS OF

Arlyn Y. Moreno Luna for the degree of Honors Baccalaureate of Science in Bioresource Research Interdisciplinary Program presented on August 27, 2013.
Title: Effects of Xanthohumol on Biomarkers of Metabolic Syndrome in Obese Rats.

Abstract Approved:__________________________________________________________
Dr. Jan Frederik Stevens

Flavonoids are a large family of compounds synthesized by plants that have a common chemical structure. It is believed that the health effects of fruits and vegetables are primarily due to dietary flavonoids content. Current research indicates that dietary flavonoids show promise for mitigating and/or preventing chronic conditions associated with metabolic syndrome. Xanthohumol (XN) is the major prenylated flavonoid of the female inflorescences (cones) of the hop plant (Humulus lupulus). Recent findings suggest that XN may impart health benefits by lowering plasma cholesterol and triglycerides level. It is important to analyze the different effects of XN on the major risk factors for metabolic syndrome such as cardiovascular disease, obesity, and type II diabetes. We examined the potential role of dietary supplementation of XN for the prevention of metabolic syndrome using the obese rat Zucker fa/fa model. The rats were fed XN using a high fat diet in the first three weeks to induce obesity, and a normal fat diet in the last three weeks. We found that XN decreases plasma glucose and insulin levels and body weight gain in obese male rats, suggesting that XN may be beneficial for preventing metabolic syndrome.

Key Words: xanthohumol, obesity, type II diabetes, flavonoids, hops
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Effects of Xanthohumol on Biomarkers of Metabolic Syndrome in Obese Rats.

by

Arlyn Y. Moreno Luna

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

________________________
Arlyn Y. Moreno Luna, Author
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Con mucho cariño esta tesis es dedicada para mis Padres

“With love, this thesis is dedicated to my Parents”
Introduction

1.1 Metabolic Syndrome

Worldwide, there has been an epidemic of obesity and diabetes. It is estimated in the U.S. that 24% of adults (20-70 years) have metabolic syndrome. The prevalence of metabolic syndrome increases with age and is over 40% for subjects over 60 years old. In addition, there is increase in the frequency of metabolic syndrome heterogeneity by sex and ethnicity [1].

Metabolic syndrome is clinically defined as having three or more of the following conditions: obesity, type II diabetes, hypertension, hyperinsulinemia, and cardiovascular disease [2]. The term, metabolic syndrome, has been used for over 80 years. Kylin, a Swedish physician, first described it in 1920s and defined it as the clustering of hypertension, hyperglycemia, and gout. In 1947, Vague correlated upper body adiposity with obesity, which was associated with metabolic abnormalities related to type II diabetes and cardiovascular disease [2].

Metabolic syndrome is also known as: the insulin resistance syndrome, syndrome X, and the deadly quartet. Some of the abnormalities of the syndrome are: type II diabetes (glucose intolerance), insulin resistance, dyslipidemia, central obesity and hypertension. Dr. Lemieux and colleagues have suggested a central component of metabolic syndrome is abdominal obesity, also known as hyper-triglyceride waist phenomenon [2].
The National Cholesterol Education Program (NCEP) and the World Health Organization (WHO) propose the most frequent definitions used in most of the research for metabolic syndrome, which include obesity, hypertension, hyperglycemia and dyslipidemia [2]. WHO’s definition requires evidence of insulin resistance and the measurement of fasting insulin or its surrogates [2].

Previous studies done in Finland by Ilanne-Parikka et al. in 2004 found that the prevalence of the metabolic syndrome using the modified WHO criteria was 14.4% in men and 10.1% in women aged 45-65 [3] Dr. Mattsson et al. in 2007 also found that metabolic syndrome was higher amongst Finnish young adult men compared with women [4]. From 1986 (0.1%) to 2001 (7.5%), the prevalence of the metabolic syndrome has increased markedly in 24-year-old adults. Overall, prevalence of the metabolic syndrome has increased amongst U.S. adults from 23.1% in 1988 to 26.7% in 1994 [2].

1.2 Obesity and Type II Diabetes

Definition of abdominal obesity varies according to population. Having an effective weight reduction improves all risk factors associated with the metabolic syndrome [2]. One of the most effective methods of weight reduction is to reduce the energy intake and increase physical activity to enhance energy expenditure. It is suggested to have a caloric intake of 500-1000 calories per day to produce a weight loss of 0.5-1.0 kg per week [2]. Currently, weight loss drugs have not been effectively tested for treatment of obesity. Bariatric surgery in the USA has increased to treat patients with morbid obesity. The effectiveness of this surgery
is 95%; most patients are no longer obese for one year after operation. However, a lot of risk factors arise post surgery such as: heart failure, atherosclerosis, heart rhythm disorders, pulmonary embolism, sleep apnea, etc. In addition, a surgery treatment only cures for fat removal and does not help to treat other diseases. Alternative treatments need to be discovered that can prevent the patient from all the described problems and have a more holistic approach. For instance, dietary flavonoids, present in fruits and vegetables, can help treat several conditions associated with metabolic syndrome including obesity and type II diabetes.

There are two main forms of diabetes; it is usually due to an autoimmune-mediated destruction of pancreatic β–cell islets, creating an absolute insulin deficiency. People with type I diabetes must take insulin to function properly and to prevent the development of ketoacidosis [5]. Type II diabetes, also known as non-insulin dependent diabetes mellitus, is characterized by insulin resistance and/or an abnormal production of insulin secretion [3]. Globally, 90% of cases are type II diabetes. People affected by type II diabetes are not dependent on exogenous insulin; however, they may require it for control of blood glucose levels if not supplied by their diet or hypoglycemic agents. A type II diabetes epidemic is taking place in both developing and developed nations. Currently, the global figures are estimated at 150 million to 220 million in 2010, and 300 million are estimated for 2025, where most cases are type II diabetes. Type II diabetes is associated with the lifestyle and obesity [5].

Currently, there are drugs in the market that can treat obesity and type II
diabetes. However most of current drugs that are synthetically made have side effects. Research needs to move toward natural components such as flavonoids to discover a possible remedy for metabolic syndrome.

1.3 Flavonoids:

Current research indicates that dietary flavonoids show promise for mitigating and/or preventing chronic conditions associated with metabolic syndrome. Flavonoids come from a large family of compounds, synthesized by plants that have a common chemical structure. Scientists have become interested in the health benefits of fruits and vegetables due to dietary flavonoids content [1]. The metabolic syndrome shows a chronic inflammatory condition, which has been involved in the onset and development of several pathological disturbances. Previous investigators have showed that various types of flavonoids have anti-inflammatory effects [6]; the impact of flavonoids on inflammation occurs via direct effects and indirect through flavonoid metabolites [7]. Xanthohumol, a prenylflavonoid, has been shown to have an anti-inflammatory effect and other health benefits from its consumption [8].
1.4 Xanthohumol:

Xanthohumol (XN) is the major prenylated flavonoid of the female inflorescences (cones) of the hop plant (Humulus lupulus) [8]. Hops are used in the brewing industry to give beer its special characteristics such as flavor and aroma. XN is the principal flavonoid present in the hop cone extracts and has a prenylated chalcone structure. Milligan et al. [9] identified 8-prenylnaringenin (8PN) as a phytoestrogen in hops. The hop flowers in the lupulin glands contain the 8-prenylnaringenin along with other prenylflavonoids and the hop acids that are the key for brewing [9]. Lupulin may reach a content of about 10% of the dry weight of the hop cone [10]. The molecular formula for XN is C_{21}H_{22}O_{5}, and molecular weight is 354.40 g/mol (Figure 1). XN is known to exert several beneficial health effects, however only few studies evaluated the safety characteristics of this natural compound.
1.4.1 Health benefits:

In Germany, hop baths were used for the treatment of gynecological disorders. Hop extracts have been reported to reduce hot flashes in menopausal women [11]. XN is reported to have anti-inflammatory, anti-oxidant, and anti-angiogenic effects. It also exhibits anti-infective activity against bacteria, viruses, fungi, and plasmodia [12]. Research showed that of the five hop chalcones tested, XN was the most effective anti-proliferative agent in human breast cancer cells (MCF-7), colon cancer (HT-29), and ovarian cancer cells (A-2780) [13]. Moreover, XN has the ability to induce apoptosis in cancer cells and to limit tumor cell invasiveness, suggesting suppression of cancer development post-initiation as well [14]. In addition, other studies suggest that inhibition of NF-κB and induction of apoptosis may be a critical mechanism by which XN acts as anti-proliferative agent in prostate hyperplasia [14].

1.4.2 Xanthohumol and Metabolic Syndrome:

Currently, there is an increase of research in functional foods to treat obesity and its complications. Hop components have been described to inhibit the metabolic activation of xenobiotics, prostaglandins, and NO production [15]. It has been shown that XN reduces lipid accumulation in 3T3-L1 adipocytes [16]. Even though there is some in vitro information about the effects of XN on lipid metabolism, there is not enough data in vivo of the effects of XN. It is important to understand the effect of oral administration of XN, using different doses and see how it affects metabolic syndrome.
MATERIALS AND METHODS

2.1 Study Design

We tested the effects of a chronic feeding of XN on metabolic syndrome using a genetic model of obesity, the Zucker obese fa/fa (ZOF) rat. In 1962, the ZOF rat was found to have an autosomal recessive mutation that prohibits the production of leptin [17]. Leptin is a hormone involved in the regulation of energy intake and energy expenditure, which includes appetite and metabolism. The basic phenomenon in the fa/fa is an error in lipid metabolism. Due to a greater excess of lipids (probably low-density lipoproteins) circulating in the blood, an excessive amount of lipids is deposited in tissues; thus, overall ZOF rats become noticeably obese at 5 weeks of age [17]. Four-week old male and female ZOF rats were purchased from Harlan (Livermore, CA, USA). Animals were housed in individual cages in temperature and humidity controlled rooms with a 12:12 on-off light cycle. All animals handling was approved by and in accordance with ethical standards of Oregon State University’s Institutional Animal Use and Care Committee (Protocol # 3689). After a two-day acclimation period, male and female animals were divided into four treatment groups (n=6/group) according to their body weight to ensure similar average body weight across groups. For three weeks of the study, all rats were maintained on a high fat (60% kcal fat) AIN-93G to induce severe obesity, then placed on a normal AIN-93G (15% kcal fat) diet for the remainder of the study. Body weight and food intake was recorded weekly.
2.2 Treatments

Four different doses of XN were administrated daily to ZOF female and male rats and were determined from previous work [18]. It was calculated using allometric scaling and an interspecies scaling factor [19, 20] for an individual weighing 64 kg (141 lb). Rats were placed into four treatment groups: control (0 mg XN /kg BW), low (1.86 mg XN/kg BW), medium (5.64 mg XN/ KG BW) and high (16.9 mg XN/kg BW).

2.3 Source Material

Animals received daily XN doses via a test meal as described [21]. XN powder (>99% pure by HPLC-UV and NMR; Anheuser Busch, St. Louis, MO, USA) was dissolved in a self-emulsifying isotropic mixture (oleic acid, Tween 80, and propylene glycol) to create various dosing solutions (0, 1.86, 5.64, and 16.9 mg XN/kg BW). The XN solutions were then added to a modified AIN-93G [22] powdered diet (corn oil replacing soybean oil) and homogeneously mixed with mortar and pestle to result in individual test meal doses (3 g). The test meal was administered after holding food for three hours to ensure complete XN consumption within 30 min. Test meals were made weekly for each treatment group based on average body weight.

2.4 Xanthohumol Analysis

Currently, mass spectrometry is an indispensible analytical tool in chemistry, biochemistry, pharmacy, and medicine. Mass spectrometry (MS) is used to
identify a specific compound from the molecular mass of its constituents. MS elucidates the connectivity of atoms within smaller molecules, identifies functional groups, determine the (average) number and eventually the sequence of constituents of macromolecules, and at times it can yield their three-dimensional structure [23]. The basic principle of MS is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and detect them qualitatively and quantitatively by their respective m/z and abundance.

Plasma levels of XN, 8PN, isoxanthohumol (IX), and 6-prenylnaringenin (6PN) were determined using the procedures and conditions detailed in earlier work [18]. Samples were analyzed with a structure elucidation to study mass-selected ions in liquid chromatography-tandem mass spectrometry (LC-MS/MS). The term tandem mass spectrometry encompasses the numerous techniques where mass-selected ions are subjected to a second mass spectrometric analysis. Samples were prepared with and without enzymatic hydrolysis to be able to determine the amount of free and conjugated XN, IX, 8PN, and 6PN in plasma.

A homogenized tissue preparation was done under these conditions: while resting on ice, liver tissue (0.500g) was homogenized in extraction solvent (MeOH-H2O, 9:1 v/v, 5ml). Samples were homogenized (30 sec) with an Omni Tissue Homogenizer (Omni TH, Omni International, Marietta, GA, USA), followed by sonication (1 min Model F60 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, USA), and centrifuged at 4000 x g for 15 min (Allegra X15R centrifuge, Beckman Coulter, Brea, CA USA). The supernatant was removed and stored as
tissue extract. The tissues extracted were prepared in duplicate. Tissue extracts underwent similar sample preparation and analysis as previously described for plasma samples. Test meals (400 mg) were analyzed by HPLC in triplicate using conditions described in [18].

2.5 Biochemical Markers

Before sacrifice, animals underwent overnight fasting. Via a cardio-puncture blood was collected and centrifuged (International Clinical Centrifuge setting 7, 10 min). Plasma was stored at -80°C until analysis. Livers were collected, flash-frozen in liquid nitrogen and stored at -80°C prior to analysis. Commercial assay kits were used to determine plasma glucose (Wako Pure Chemicals, Richmond, VA), triglycerides, cholesterol (Thermo Scientific, Middletown, VA). Insulin was assayed by ELISA (Alpco Insulin RAT ELISA, Alpco Diagnostics, Salem, NH) according to the manufacturer’s directions.

In the glucose assay, α-D-glucose found in the plasma sample is first converted to the β-isomer by mutarotase, and is then oxidized by glucose oxidase to produce hydrogen peroxide (H$_2$O$_2$) as one of the products. H$_2$O$_2$ reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to produce a red pigment. This red pigment present in the sample is read on a 96 well plate reader at 505 nm. Quantification was done with a standard curve of increasing concentrations of glucose that was also measured along with the samples.

In the triglycerides assay, triglycerides present in the sample are hydrolyzed by a lipase to free fatty acids and glycerol. The glycerol is phosphorylated by glycerol
kinase to produce glycerol-3-phosphate, which is then oxidized by
glycerolphosphate oxidase forming \( \text{H}_2\text{O}_2 \) as one of the products. \( \text{H}_2\text{O}_2 \) reacts with
4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzene sulfonate to produce a
red-colored dye, which is then read at 550 nm.

In the cholesterol assay, the cholesterol esters are hydrolyzed by cholesterol
esterase to free cholesterol. Free cholesterol is then oxidized to cholest-4-en-3-
one and \( \text{H}_2\text{O}_2 \) by cholesterol oxidase. \( \text{H}_2\text{O}_2 \) reacts with hydroxybenzoic acid and
4-aminoantipyrine to produce the quinoneimine dye, which is read at 550 nm.

### 2.6 Preparation of Liver Protein Extracts for Western Blotting

Liver tissue samples (100-150 mg) were homogenized in homogenization buffer
(HB) (1-1.5mL) using an OMNI homogenizer. HB was prepared as described in
[24] and consisted of 20 mM Hepes, 50 mM disodium glycerol 2-phosphate, 2
mM Na EDTA, 1 mM sodium orthovanadate, 1 % Triton X-100, 10% glycerol, 2
mM DTT, and protease inhibitors (Protease Inhibitor Cocktail (Cat no P8340),
Sigma-Aldrich, St. Louis, MO, USA). Liver homogenates were incubated (30 min,
4°C) to solubilize proteins. After incubation, homogenates were centrifuged
(12,000 x g, 20 min, 4°C) and supernatant was removed. Samples were
analyzed for total protein content using Coomassie Plus Protein Assay Reagent
(Pierce cat. no.23236, Rockford, IL) according to the manufacturer’s directions.
2.6 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blotting

Liver extracts were mixed with Laemmli sample buffer (Bio-Rad cat. no. 161-0737, Hercules, CA) with 2-mercaptoethanol (5%) and heated (100°C, 5 min) before cooling to room temperature. The protein samples were loaded onto a 10-well 10% Tris-glycine precast gel (Bio-Rad cat. no. 456-1033) and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to nitrocellulose membranes by electroblotting. The membranes were blocked by incubating in 5% non-fat dry milk dissolved in Tris buffered saline with 0.1% Tween 20 (TBS-T). After washing with TBS-T, the membranes were incubated with a primary antibody and a secondary antibody (goat anti-rabbit IgG conjugated with horseradish peroxidase, Bio-Rad). The primary antibodies (Cell Signaling Technology, Danvers, MA) used were raised in rabbits against AMPK, phosphorylated AMPK (pAMPK), Akt, phosphorylated Akt (pAkt), and β-actin. Protein bands were detected by incubating the nitrocellulose membranes with a chemiluminescence substrate (SuperSignal West Pico Chemiluminescent substrate, Thermo cat. no. 34080) and exposing the membranes to X-ray film.
2.7 Statistics

The data was analyzed using Statistical Analysis Software (SAS) (Version 9.1, SAS Institute, Cary, NC) or GraphPad InStat (GraphPad Software, La Jolla, CA). A data point for insulin that was more than four standard deviations from the mean of the group average was excluded from further statistical analysis. The treatment effects on body weight were assessed using repeated measure analysis over time (PROC MIXED procedure). Effects on plasma biochemical markers were determined by Student t-test or one-way ANOVA and post-hoc test using LSD or Dunnett's test. The significance level was set at p<0.05.
Results and Discussion

3.1 Body Weight

In our study, we analyzed the effects of XN consumption on body weight. A total of 48 ZOF rats were observed for a total of six weeks. In our study, we discovered that the supplementation of XN reduced body weight in male rats. This effect was seen in male high XN group. Comparing the high treatment to control group there was a significant decrease (p<0.05) in their body weight. We also observed lower body weight among the other XN treatment groups both in males and females; to the control group, but this was not significant (Figure 2). Previous research has demonstrated that consumption of dietary soy isoflavones decreases body weight and abdominal white adipose tissue, similar to our findings with XN [25]. Also, a trend was observed in female rats for a dose dependent effect of XN on body weight (Figure 2). Perhaps, this observation of lower body weight in female rats can become statistically significant if we increase the number of the rats per treatment group or duration of XN supplementation.
Figure 2. Mean body weight of male and female obese Zucker fa/fa rats (n=6/group) receiving various daily doses (0, 1.86, 5.64, 16.9 mg/kg BW) of xanthohumol for 6 weeks. Statistical difference from the control group is denoted by the * (p < 0.05). All values are expressed as mean ± SE.
3.2 Plasma Glucose

There are several classes of medications to lower plasma glucose in metabolic syndrome or in Type 2 diabetes. They include sulfonylureas (increases insulin secretion by the pancreas), acarbose (inhibitor of intestinal alpha-glucosidase), metformin (inhibitor of hepatic gluconeogenesis), and rosiglitazone (activator of PPAR-γ in fat and muscle). Inhibitors of SGLT1 and SGLT2 such as LX4211 lowers plasma glucose by inhibiting the reabsorption of glucose by the kidneys [26]. Glucagon-like peptide-1 (GLP-1 secreted by intestinal cells) agonists (or incretin mimetics) such as Exenatide also lower plasma glucose by stimulating insulin secretion while suppressing glucagon secretion [27]. Glucagon, as opposed to insulin, increases plasma glucose by stimulating the conversion of liver glycogen into glucose. However, these drugs have certain undesirable side effects prompting us to investigate natural products such as XN to supplement or replace existing therapies for lowering blood glucose in metabolic syndrome or in Type 2 diabetes.

Low and medium XN treatment did not exhibit effects on plasma glucose. However, when comparing the high XN treatment group to the control male group, there was a significant decrease (p<0.05) in plasma glucose levels (Figure 3) but not in female rats. This also corresponds to other research, with the consumption of soy or soy isoflavones decreasing plasma LDL levels in males but not in female hamsters [28]. Past research has shown that XN feeding for four weeks caused a decrease in plasma glucose in obese male mice [29]. A plasma glucose level is the amount of glucose (sugar) present in the blood.
Naturally each organism regulates blood glucose levels as part of the metabolic homeostasis. Primary glucose is the source of energy for the body's cells, and blood lipids. Glucose is transported from the intestines or liver to body cells via bloodstream and is available for cell absorption mediated by insulin produced primarily in the pancreas. Future research needs to be done to examine the mechanisms why females do not have the same response as males (Figure 3).

**Figure 3.** Mean plasma glucose levels of male and female Zucker fa/fa rats (n=6/group) receiving various daily doses (0, 1.86, 5.64, 16.9 mg/kg BW) of xanthohumol for 6 weeks. Statistical difference from the control group is denoted by (p < 0.05). All values are expressed as mean ± SE.
3.3 Plasma Insulin, Triglycerides and Cholesterol

Insulin is a peptide hormone produced in the pancreas by beta cells and is a regulator of carbohydrate and fat metabolism in the body. Insulin in the body causes cells of the liver, skeletal muscles and fat tissues to absorb glucose from the blood. Once in the liver and skeletal muscles, glucose is stored as glycogen, and it is stored as triglycerides in fat cells. Cholesterol is considered within the class of lipid molecules. As biomarkers of metabolic syndrome, we analyzed insulin, triglycerides and total cholesterol levels in the plasma of control and XN-treated rats. Plasma triglycerides (Figure 4A) and total cholesterol (Figure 4B) were not affected by XN treatment. However, plasma insulin levels were significantly decreased in male rats treated with medium- and high-dose XN but not in females (Figure 4C). The reduction in plasma glucose in the high-dose XN male group may be explained in part by alleviating the hyperinsulinemia or insulin resistance in these obese male rats. The control male rats had much higher levels of plasma insulin as compared to the control female rats (Figure 4C). Plasma cholesterol was also significantly higher in control males than in control females (Figure 4B).

Although there was no effect of high XN on the triglycerides levels in male rats in our study, Kirkwood et.al. [30] found that XN decreased fasting plasma dicarboxylic acids and medium-chain acylcarnitines in these male rats, suggesting that XN alleviates dysfunctional lipid oxidation. These metabolites are considered markers of dysfunctional lipid metabolism or dysfunctional mitochondria. Fatty acids are transported across the mitochondrial membrane as
acylcarnitines, which are produced by the reaction of fatty acids with carnitine mediated by carnitine acyltransferases such as carnitine palmitoyltransferase 1 (CPT1). Acylcarnitines become elevated in plasma when there is incomplete fatty acid oxidation because of mitochondrial dysfunction. Therefore, using total plasma triglyceride levels alone may not be a satisfactory biomarker for examining the beneficial effects of XN on dysfunctional lipid metabolism.
Figure 4. Mean plasma triglyceride (A), cholesterol (B) and insulin (C) levels of male and female obese Zucker fa/fa rats receiving various doses (0, 1.86, 5.64, 16.9 mg/kg BW) of xanthohumol for 6 weeks. A data point for insulin that was more than four standard deviations from the mean of the group average was excluded from further statistical analysis. All values are expressed as mean ± SE.
3.4 Western Blotting AMPK and Akt

To understand the mechanisms by which XN decreases plasma glucose levels in male rats, we tested for protein levels of AMPK, pAMPK, Akt, and pAkt in male rat livers by Western blotting. The enzyme 5’ adenosine monophosphate-activated protein kinase (AMPK) plays a role in cellular energy homeostasis [31]. It is expressed in different tissues such as the liver, brain, and skeletal muscle. The main role of AMPK activation is the stimulation of hepatic fatty acid oxidation and ketogenesis, improved binding of insulin to insulin receptors, inhibition of cholesterol synthesis, lipogenesis and triglycerides synthesis, inhibition of adipocyte lipolysis and lipogenesis, inhibition of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells. Activation of AMPK (phosphorylation of AMPK) leads to suppression of glucose production by the liver (gluconeogenesis) through increased expression of SHP which in turn inhibits the expression of the hepatic gluconeogenic genes PEPCK and G6Pase [32]. A serine/threonine protein kinase (Akt) that is a member of the AGC kinase family (PKA, PKC, PKG), also known as Protein Kinase B (PKB), is a protein kinase that plays a role in multiple cellular processes like glucose metabolism, and apoptosis [33]. The phosphorylation of Akt can affect numerous downstream targets such as insulin activation, survival, protein synthesis and proliferation. Additional research needed to find the possible link between AMPK activation by XN supplementation. In the ApoE- deficient mouse model, supplementation of XN increases the activation hepatic AMPK which could be due to inhibition of Akt
[33]. In our present study, XN treatment on male rats did not produce a consistent reduction in the levels of liver Akt (Figure 5B). However, XN treatment of male rats unexpectedly decreased the levels of liver AMPK (Figure 5A). Unfortunately, the effects of XN on the levels of pAMPK and pAkt could not be evaluated because the Western blots did not detect any bands for these protein targets. This could have been due to the antibodies used, or that in our rat model, phosphorylated forms of AMPK and Akt are undetectable. In the Apo-E mouse model, pAMPK was also not detected by Western blotting in controls but were markedly increased by XN treatment [33]. Further work needs to be done to confirm the effects of XN on the activation of AMPK and Akt in the rat obese model using mass spectrometry to detect phosphorylated AMPK and phosphorylated Akt.

![Figure 5](image)

**Figure 5.** AMPK (A) and Akt (B) levels in liver samples of obese male rats as determined by Western blotting.
CONCLUSIONS

In this study, we analyzed the effects of dietary XN supplementation with on components of metabolic syndrome using an obese rodent model Zucker fa/faqerats. XN was found to reduce body weight, plasma glucose and plasma insulin levels in male, but not in females rats. There was no effect of XN on plasma triglyceride and total cholesterol levels in both males and females. Overall, our study has shown that ingestion of XN through the diet for six weeks in obese male rats showed a beneficial effect on metabolic syndrome. Further research needs to be done with human subjects to test for any side effects. Also, further research is needed to understand the mechanism behind XN affecting male rats but not female rats.
BIBLIOGRAPHY


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