

**HEALTH EFFECTS OF *MORINDA CITRIFOLIA* (NONT)
ON LIPID AND GLUCOSE METABOLISM**

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ABSTRACT

The relationship between metabolic abnormalities including obesity, insulin resistance, hypertension, and dyslipidemia is growing at an alarming rate in the United States. Although there are many conventional treatments for these metabolic disorders, many people are turning to complementary and alternative medicine. *Morinda citrifolia* (noni) has been used as a folk remedy for centuries by Polynesian healers. Recently there has been a dramatic increase in the interest and use of this plant. Despite the purported health benefits of consuming noni juice, there have been very few scientific studies validating or refuting these claims. The aim of our study was to investigate the mechanism underlying the beneficial effects of noni juice *in vitro* and *in vivo* with focus on glucose and lipid metabolism. Human hepatoma cell (HepG2) treated with 1.5% and 3% (v/v) noni juice was shown to reduce apolipoprotein (apoB) secretion, cellular triglyceride, and cholesterol mass. However, when murine pre-adipocytes (3T3-L1) were treated with noni during differentiation (1, 2, and 3% v/v), there was an increase in lipid accumulation and lipolysis. Short term (5 weeks) treatment with noni (1.5 μ l/g body weight, twice daily) in male C57BL/6J mice fed a control diet or high fat diet exhibited significant improvements in glucose regulation and reduced weight gain. However, there were no significant changes in lipid metabolism and

adiponectin secretion. Long term (12 weeks) treatment with noni also showed significant improvement in glucose regulation, and slight modifications in lipid metabolism. On the other hand, there were no significant changes in adipokine and apolipoprotein secretion, as well as peroxisome proliferator activated receptor gamma (PPAR γ) expression. Based on the data obtained from these studies, it is evident that the noni juice may be beneficially modulating glucose metabolism. However, the molecular mechanism on which noni juice is acting must still be elucidated. Also, there are some indications that noni juice may affect lipid metabolism *in vitro* and *in vivo* but further studies must be conducted to conclusively determine the effects.

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LIST OF ABBREVIATIONS

| | |
|----------------|---|
| μg | microgram |
| μl | microliter |
| ApoA1 | apolipoprotein A1 |
| ApoB | apolipoprotein B 100 |
| ATP III | Adult Treatment Panel III |
| BMI | body-mass index |
| BSA | bovine serum albumin |
| BW | body weight |
| CAM | complementary and alternative medicine |
| CH | cholesterol |
| CJ | commercial noni juice |
| Con | control |
| cox | cyclo-oxygenase |
| CVD | cardiovascular disease |
| DMEM | Dulbelco's Modified Essential Medium |
| DMSO | dimethyl sulfoxide |
| ECL | enhanced chemiluminescence |
| EDTA | ethylenediaminetetracetic acid |
| FBS | fetal bovine serum |

| | |
|--------------|---|
| FDA | Food and Drug Administration |
| FFA | free fatty acid |
| g | gram |
| GTT | glucose tolerance test |
| h | hour |
| HDL | high density lipoprotein |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HFD | high fat diet |
| HJ | homemade noni juice |
| HRP | horseradish peroxidase |
| i.p. | intraperitoneal |
| IACUC | Institutional Animal Care and Use Committee |
| IgG | immunoglobulin G |
| ITT | insulin tolerance test |
| KCl | potassium chloride |
| kg | kilogram |
| L | liter |
| MEME | minimum essential medium eagle |
| mg | milligram |
| mM | millimolar |
| MTP | microsomal transfer protein |

| | |
|--------------------------------|---|
| NaCl | sodium chloride |
| NaOH | sodium hydroxide |
| NEFA | non-esterified free fatty acid |
| NJ | noni juice |
| PBS | phosphate buffered saline |
| PPARγ | peroxisome proliferator activated receptor |
| SD | standard deviation |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel |
| T2D | type 2 diabetes |
| TG | triglyceride |
| Tris-HCl | trizma-hydrochloride |
| v/v | volume to volume |
| VLDL | very low density lipoprotein |
| x g | gravitational force |

CHAPTER 1

INTRODUCTION

General Introduction

Morinda citrifolia, more commonly known as noni is a small evergreen plant said to have originated from Southeast Asia, and distributed to the Western Pacific Islands by humans or other sources (Morton, 1992). Other common names for the plant include, but are not limited to morinda, Indian mulberry, nona, nonu, and cheese fruit. It grows in tropical areas of the South Pacific, including Australia, Malaysia, the West Indies, India, Vietnam, the Philippines, Taiwan, and Hawaii. Noni has been used medicinally for centuries as a panacea, or cure-all. The seeds, leaves, bark, and roots were all used by healers who were versed in the properties of this plant.

The first noni product was brought to Hawaii's commercial market in 1992 by Herbert Moniz. This product was a capsule filled with dehydrated noni powder (US patent 5288491, Moniz, 1992). During this decade, the noni juice became increasingly popular as a health drink. This burst in popularity began when David Marcus, of Hawaiian Herbal Blessings, Inc., began internationally marketing this juice as an herbal remedy. There are now hundreds of companies marketing noni juice and noni products as a complementary and alternative medicine (CAM) though there are very few scientific studies validating these health benefits.

Plant Description

Noni belongs to the family Rubiaceae and genus *Morinda*. *Morinda* contains more than 80 species; the most well known of these being *Morinda citrifolia*. The noni plant is a small evergreen shrub that flowers through out the year. It has large glossy, elliptical leaves with pinnate veins (McClatchey, 2002). The plant has seeds that contain air chambers allowing them to float across long distances in the ocean (Krauss, 1993). This is said to be one of the modes of transportation and dispersal of the plant and its arrival to coastal zones (Morton, 1992). The seeds of the noni tree are found in the fruit, which are ovoid in shape, and sprout small white tubular flowers. They grow several inches in length and ripen from a deep green to a pale yellow. As the fruit ripens, its skin turns translucent and begins to thin. The pulp of the fruit slowly breaks down and seeps through the skin, producing a pungent odorous liquid.

Chemical Constituents of Noni Fruit

Identification of compounds found in the noni plant has been examined by several scientists. Many of these constituents have been identified through different extraction methods and analyses. The chemical compounds found in the plant are listed in the following table.

TABLE 1.1*Compounds Found In the Noni Fruit*

| Noni Plant Part | Compounds | Reference |
|------------------------|------------------------------|------------------|
| Fruit and Fruit Juice | <u>Fatty Acids</u> | |
| | Octanoic acid | (Farine, 1996) |
| | Palmitic acid | |
| | Octadecanoic acid | |
| | Hexanoic acid | |
| | <u>Vitamins and Minerals</u> | |
| | Potassium | (Morton, 1992) |
| | Vitamin C | (Dixon, 1999) |
| | Calcium | |
| | <u>Phenolic Compounds</u> | |
| | Scopoletin | (Farine, 1996) |
| | <u>Alkaloids</u> | |
| | Xeronine | (Heinicke, 1995) |
| | <u>Volatile Compounds</u> | |
| | Organic acids | (Farine, 1996) |
| | Alcohols | |
| | Esters | |
| Ketones | | |
| Lactones | | |

TABLE 1.2*Compounds Found in the Noni Plant*

| Noni Plant Part | Compounds | Reference |
|------------------------|-------------------------------------|------------------------|
| Leaves | <u>Sterol Derivatives</u> | |
| | β -sitosterol | (Ahmad and Bano,1980) |
| | <u>Anthraquinones</u> | |
| | Damnacanthal | (Ahmad and Bano,1980) |
| | <u>Vitamins and Minerals</u> | |
| | Vitamin A | |
| | <u>Glycosides</u> | |
| | Iridoid glycosides | (Wang, 1999) |
| | Flavanoid glycosides | (Sang, 2001) |
| | Citrifolin A | (Sang, 2001) |
| Roots | <u>Anthraquinones</u> | |
| | Damnacanthal | (Bowie and Cook, 1989) |
| | Morindone | |
| | Rubiadin | |
| Flower | <u>Glycosides</u> | |
| | Flavonoid glycosides | (Tiwari, 1977) |
| | Anthraquinone glycoside | |
| Seed | <u>Fatty Acids</u> | |
| | Linoleic acid | (Daulatabad, 1989) |

Medicinal Properties of Noni

There are a wide range of claims that promote the noni a plant as a medicine for cancer, allergies, arthritis, asthma, chronic fatigue syndrome, circulation, constipation, diabetes, fibroid tumors, heart (arteries and cholesterol), high blood pressure, kidney infections, sleep apnea, vitality, menstrual regulation, weight loss, and immune system regulator. Most of the evidence for the health benefits of noni is from testimonial accounts and many of these claims are not verified with scientific evidence. However, there are some uses of the noni plant such as the anti-microbial and anti-cancer properties that have been investigated substantially. Other studies conducted on the traditional medicinal properties of the noni plant include the effects of noni on cardiovascular disease, inflammation, and diabetes.

Anti-Cancer Effects of Noni

The research team of Hirazumi and Furusawa (1999) and Hirazumi et al. (1996) showed an ethanol precipitated fraction of noni juice to modulate the immune system and exhibit anti-tumor effects against Lewis lung carcinoma (LLC). They showed this by inoculating mice with LLC and treating them daily with noni juice. The mice consuming 15 mg of noni juice a day had a 119% increase in life span, and ingestion of the precipitated fraction of noni combined

with conventional chemotherapy also increased the life span of the mice. In cell culture models, the production of T-cells, macrophages, and thymocytes were stimulated by the noni precipitated fraction (Hirazumi et al, 1996 and Hirazumi and Furusawa, 1999). These cells are important in the generation of cytokines that mediate tumor cytotoxicity and cytotoxicity. The noni precipitate also displayed some stimulatory effects on the release of cytokines from murine effector cells that slow down tumor cell cycle, activate macrophage activity, and increase response of cells to fight the growth of the tumor, leading to tumor death (Hirazumi et al, 1996 and Hirazumi and Furusawa, 1999). Another group studying the effect of noni juice on cancer *in vivo* found that a commercial noni juice was able to prevent the formation of chemical carcinogen-DNA-adduct (Wang & Su, 2001). The rats in this study had cancer induced artificially in explicit organs and were treated with 10% noni juice in their food and water, ad libitum for one week. They found that depending on the sex and the organ there were differentially modulated DNA-adduct formation, with reduction in the females, by 30%, 42%, 41%, and 80% in the heart, liver, lungs, and kidneys, respectively. Whereas males showed 60%, 70%, 50%, and 90% in the heart, liver, lungs, and kidneys respectively (Wang & Su, 2001)

Anti-Microbial Effects of Noni

Studies have shown that noni inhibits the growth of certain bacteria,

including *Staphylococcus aureus*, *Pseudomonas aeruginos*, *Proteus morgaii*, *Bacillus Subtilis*, *Escherichia coli*, *Heliobacter pylori*, *Salmonella* and *Shigella* (Atkinson, 1956). The phenolic compounds (listed in Table 1.1) present in the plant may be responsible for these anti-microbial effects. Reports by Saludes et al. (2002) showed that ethanol and hexane extracts of the noni inhibit *Mycobacterium tuberculosis* growth by 89-95%. Thus, the use of noni as an anti-microbial agent may provide some beneficial properties as CAM.

Anti-inflammatory Effects of Noni

One study showed that commercial noni juice had a selective inhibition effect on some cyclo-oxygenase enzymes (cox1 and cox2) that are important in the inflammation process (Su *et al.*, 2001). In this study, a comparison between noni juice and commercial non-steroidal inflammatory drugs such as aspirin, indomethacin, and celebrex was tested for the ability to inhibit the activity of the cox enzymes. Noni juice was able to inhibit cox enzyme activity *in vitro*, and had strong anti-inflammatory effects comparable to celebrex. Li et al. (2003) demonstrated that an ethanolic noni fruit powder extract had over 70% inhibition of cox1 whereas the ethanolic extraction of the bark and fresh fruit juice had only a 27 and 38% inhibition.

The aqueous extract of noni juice was also shown to have some anti-inflammatory effects. When a locally acute inflammatory response was induced in

rats with bradykinin (pro-inflammatory agent), oral administration of noni extract exhibited rapid inhibition of rat paw edema. The result is suggested to be due to the interference with the bradykinin B2 receptor mediated mechanism by which bradykinin induces rat paw edema (McCoy *et al.*, 2002).

Inhibition of LDL-oxidation by Noni

Atherosclerosis development is closely associated with the oxidation of low density lipoproteins (LDL), which occurs in the presence of free radicals. The oxidized LDL is taken up by macrophages and eventually accumulates large amounts of lipids and converts into foam cells. These foam cells are responsible for the fatty streaks on arteries and cause atherosclerosis. Salleh *et al.* (2002) tested the leaves of the noni plant for the ability to inhibit copper induced LDL oxidation and found that it only inhibited oxidation by 5%. However, Kamiya *et al.* (2004) demonstrated copper-induced LDL oxidation was inhibited by 88 and 96% in the presence of a methanol and ethyl acetate extract, respectively. Therefore, the use of noni juice extracts may be useful as a CAM in the prevention of cardiovascular disease (CVD).

Anti-diabetic Properties of Noni

High levels of circulating blood glucose after an overnight fast often indicates problems with glucose metabolism. Measurements of fasting blood glucose levels are important in the diagnosis of insulin resistance and diabetes.

Recent studies by Nayak et al. (2007) found that oral administration of noni juice in streptozotocin-induced diabetic rats had significantly reduced fasting blood glucose levels. They implicated that the reduction in glucose levels aided in the increased rate of healing, a problem often found in diabetic patients.

Complementary and Alternative Medicine

The use of herbal therapies are considered to be an essential part of cultural medicine that has been passed down from generation to generation (Vickers & Zollman, 1999). The use of CAM in the United States has increased by 40-50% within the past 10 years (Barnes *et al.*, 2004), and has a reputation among health conscious consumer for being useful (Vickers & Zollman, 1999). In the US over \$34 billion per year are spent on CAM therapies and are costs not covered by conventional health care financing systems (Linde *et al.*, 2001)

Metabolic syndrome

Metabolic risk factors, such as abdominal obesity, hypertension, dyslipidemia, and insulin resistance or glucose intolerance occurring together in a single person is often termed metabolic syndrome. It is estimated that metabolic syndrome occurs in about 20% to 25% of the U.S. population (Dunstan *et al.*, 2002). The likeliness to have a heart attack or stroke is twice as high as those

without metabolic syndrome (Isomaa *et al.*, 2001). There is such a vicious cycle of metabolic abnormalities. The prevalence of obesity, type 2 diabetes (T2D) and CVD are so greatly overlapped that it has been gradually more evident that people with obesity are likely prone to T2D, and vice versa, ultimately proving to be a major risk factor for CVD (Rana *et al.*, 2007).

Obesity, Dyslipidemia, and Insulin Resistance

Over the past few decades, obesity rates have surged toward epidemic proportions. Obesity has increased by 61% from 1991 to 2000 (Mokdad *et al.*, 1999) afflicting 19.8% of the United States adult population in the year 2000 (Mokdad *et al.*, 2000). Obesity is characterized by an excessive amount of body fat or adipose tissue in relation to lean body mass. Measuring a person's body mass index (BMI) is the most common way of determining obesity. Excess energy intake can lead to the body's inability to function properly, resulting in obesity. This disease is endocrine in nature and can lead to complications, including T2D, coronary artery disease, hypertension, and dyslipidemia (Guri *et al.*, 2006)

Dyslipidemia is characterized by increased levels of very low-density lipoprotein (VLDL), small dense LDL particles, triglycerides, cholesterol and total cholesterol, and a decrease in high density lipoprotein (HDL) cholesterol levels. The dysregulation of these lipids are predictive for CVD endpoints.

The synthesis of cholesterol occurs in virtually all tissues, the liver being one of the largest contributors to the cholesterol pool (Siperstein *et al.*, 1952). Regulation of cholesterol synthesis and secretion is important for the maintenance of circulating levels in the body and is regulated heavily by the liver and is needed for many essential functions in the body (Goldstein & Brown, 1990, Maxfield & Tabas, 2005). Too much cholesterol in the plasma can cause cardiovascular disease and the secretion of cholesterol into the bile may result in cholesterol precipitation in the bile duct and gall bladder (Zanlungo & Nervi, 2003). The liver is also an important sight for the secretion of apolipoproteins, which are packaged with triglyceride, cholesterol and cholesterol esters, and phospholipids. Once secreted, it is transported to peripheral tissue for further use.

Triglycerides can not form stable micelles by themselves because they are only slightly soluble in water. Thus, they coalesce within adipocytes as lipid droplets that serve as major energy reserves for the body. To mobilize the stored triglycerides in the adipose tissue, lipases hydrolyze the triglycerides releasing fatty acids and glycerol (Langin, 2006). Once glycerol is released, it is transported through the blood to the liver where it can be phosphorylated and used to reform triglycerides. Free fatty acids are released to the plasma and bind to albumin. It is then carried to other tissues where it can diffuse into cells and be used for energy.

The occurrence of insulin resistance is often associated with the previously mentioned clinical disorders. Insulin resistance occurs when target cells fail to

respond to ordinary levels of circulating insulin, and as a result, is unable to provide normal glucose and lipid homeostasis. Therefore, in order to maintain normoglycemia higher than normal concentrations of insulin are needed. Insulin resistance in adipocytes (fat cells) results in lipolysis of stored triglycerides, which is secreted into the blood as free fatty acids and glycerol (Eckel *et al.*, 2005, Wang *et al.*, 2004).

Role of Adipose Tissue in Lipid and Glucose Homeostasis.

The adipose tissue depots release large amounts of FFA and secrete adipocytokines that impact glucose and lipid homeostasis. Free fatty acids secreted from adipocytes are primarily released during fasting to supply the body with fuel for energy and circulating FFAs promote hepatic glucose output and inhibit adipose and muscle glucose uptake (Roden *et al.*, 1996, Roden *et al.*, 2000). The increased availability and utilization of FFA play a crucial role in the development of insulin resistance and in insulin resistant states, the adipose tissue does not respond to the anti-lipolytic effect of insulin and as a consequence, releases excessive amounts of FFAs. (DeFronzo *et al.*, 1985).

Adipocytokines participate in the development of insulin resistance, but may also modulate endothelial dysfunction, and promote atherosclerosis (Lau *et al.*, 2005). Adipocytokines appear to be at the interface of metabolic diseases, and PPARs have been demonstrated to have important effects on their expression.

PPAR γ is a ligand activated nuclear transcription factor. It is primarily located in the adipose tissue where it serves as an essential regulator of adipocyte differentiation (Rosen *et al.*, 1999, Tamori *et al.*, 2002). PPAR γ have been demonstrated to have important effects on the expression of adipocytokines.

Adiponectin is one of these adipocytokines that is exclusively secreted by adipocytes and is tightly controlled (Trujillo & Scherer, 2005). It has a major role in improving insulin sensitivity through its actions in the liver, where it decreases lipid synthesis, decreases uptake of FFAs, and aids in the repression of gluconeogenesis (Combs *et al.*, 2001, Kershaw & Flier, 2004, Meier & Gressner, 2004). Increased adiponectin levels have been associated with decreased risk of T2D and obesity (Chandran *et al.*, 2003, Kinlaw & Marsh, 2004) whereas the decrease in adiponectin is associated with metabolic syndrome (Ryo *et al.*, 2004). Adiponectin has been associated with dyslipidemia, where there is a negative correlation with circulating triglyceride and a positive correlation with circulating HDL cholesterol (Kazumi *et al.*, 2004, Matsubara *et al.*, 2002) The decrease in adiponectin levels is also associated with an increase in smaller LDL particle size (Hulthe *et al.*, 2003). Adiponectin synthesis is impaired in obesity and may be associated with leptin resistance or deficiency. Adiponectin was also shown to lower plasma triglycerides and FFAs as well as reverse insulin resistance and hyperglycemia (Yamauchi *et al.*, 2001). It has been shown that mice lacking adiponectin had delayed clearance of FFA from the plasma, had elevated blood

glucose, and suffered from insulin resistance when fed a high fat diet (Maeda *et al.*, 2002).

Leptin is also a cytokine secreted from adipocytes. In obese individuals there are usually elevated leptin levels due to the enlarged adipose mass. Circulating leptin acts as a satiety sent to the hypothalamus and to target tissues (Bjorbaek & Kahn, 2004). Leptin is important for increasing energy expenditure and decreasing food intake, which indirectly promotes insulin sensitivity (Webber, 2003) and has been shown to aid in normal insulin sensitivity (Oral *et al.*, 2002, Yamauchi *et al.*, 2001). It has also been shown that administration of leptin improves insulin sensitivity (Oral *et al.*, 2002).

Resistin is another cytokine secreted from adipose tissue and have been shown to be elevated in many murine models of obesity. Resistin has been implicated in roles in reducing glucose uptake, impairing glucose tolerance, and insulin action (Steppan *et al.*, 2001). When resistin is neutralized in the blood, it improves circulating glucose levels and insulin sensitivity in mice with dietary obesity, and also promotes glucose uptake by adipose tissue, suggesting that it may contribute to the relationship between obesity and diabetes (Steppan *et al.*, 2001). It has been shown that mice lacking resistin have reduced glucose production in the liver. When mice have diet induced obesity, the absence of resistin decreases fasting blood glucose levels compared to wild type mice on the same high fat diet (Banerjee *et al.*, 2004).

Hypothesis and Objectives

The ancient folklore of the noni plant as a cure all medicinal substance has lead scientist to study the various claims. Many of these claims are still unsubstantiated with scientific evidence and these purported health benefits are highly based on testimonial accounts. There have been positive studies showing the reduction of LDL oxidations (Kamiya *et al.*, 2004) as well as the improved blood glucose in diabetic induced rats (Nayak *et al.*, 2007). Based on the previous studies, we **hypothesize** that the consumption of noni juice will exhibit some beneficial health effects in alleviating symptoms of metabolic syndrome. Therefore, the **objective** of this study was:

- (i). Examine the short term *in vivo* effects of commercial noni juice on lipid and glucose metabolism in C57BL/6J mice treated for 5 weeks.

- (ii). Examine the *in vitro* effects of commercial noni juice on lipid synthesis and secretion in HepG2 and 3T3-L1 cell culture models.

- (iii). Examine the long term *in vivo* effects of commercial noni juice and homemade noni juice on lipid and glucose metabolism in C57BL/6J mice treated for 12 weeks.

CHAPTER 2

HEALTH EFFECTS OF NONI JUICE IN VIVO USING C57BL/6J MICE

Introduction

The incidence of obesity in the United States has increased rapidly in part due to the sedentary lifestyle and increased caloric intake. This rise in obesity predisposes the population for metabolic syndrome. Previous studies conducted by other labs have shown noni consumption to have some beneficial effects in improving cardiovascular health, inflammation, and diabetes. However, in these studies, the regulation of lipid and glucose metabolism was not examined. In diet-induced obese states, dyslipidemia and impaired glucose tolerance is usually prevalent. Many studies have been conducted on lipid and glucose metabolism in diet-induced obese C57BL/6J mice. Very little is known about the effects of noni on the lipid profiles, which is an important factor in metabolic syndrome. Therefore, the aim of this study was to investigate the effect of commercial noni juice on lipid and glucose profiles in the C57BL/6J male mouse fed a high fat diet (HFD).

Materials and Methods

Animal Experiments. The animal protocol was approved by the Institute of Animal Care and Use Committee (IACUC) at the University of Hawaii at Manoa and experiments were conducted according to their guidelines. 6 week old male C57BL/6J mice (Jackson Laboratory, Wilmington, MA), weighing 19.3 ± 1.15 g were housed individually. The mice were maintained in a temperature controlled room at 20°C to 22°C and kept on a 12h light/12h dark cycle. Food and water were available ad libitum.

Mice were divided into 4 groups (n=5), control diet (Con), Con + Noni Juice (Con + NJ), high fat diet (HFD), and HFD + NJ. Con mice received chow containing 11 kcal % fat and sucrose while HFD mice received chow containing 58 kcal % fat and sucrose. The noni treated groups received treatment of commercial noni juice at human consumption levels of about 3 oz. (90 ml) twice a day, which equates to 1.5 μ l of noni juice per gram of body weight twice daily (<http://nonipower.blogspot.com/2006/04/noni-dosage.html>, <http://www.noni-is-good-for-you.com/>).

The commercial noni juice was purchased from a local health store. The ripe noni fruit are harvested, washed, and dried. The fruit are packed into tanks and left outside. The juice slowly seeps out of the fruit and ferments for about 6-8 weeks. The juice is drained from the tanks, pasteurized (heated to 180 degrees), and bottled for sale.

Animal Measurements. Body weight and food intake was measured daily. Fasting glucose levels were measured from tail vein bleed of mice fasted for 4h using one touch ultra meter and glucose strips (LifeScan, Milpitas CA) at end of 4 weeks. Glucose tolerance tests (GTT) was measured after a 4h fast from tail vein bleed of mice injected intraperitoneally (i.p.) with 2 mg/g body weight of dextrose solution (Phoenix Pharmaceutical, St. Joseph MO) after 4 weeks of treatment. Insulin tolerance tests (ITT) was measured after a 4h fast from tail vein bleed of mice injected (i.p.) with or 0.75 units/kg body weight of insulin (Novolin R, Novo Nordisk Pharmaceuticals, Princeton NJ) after 4 weeks of treatment. Animals were fasted overnight with free access to water and were anesthetized with isoflourane. Blood was collected by cardiac puncture under anesthesia at the start of sacrifice. Tissue was excised, weighed and immediately snap frozen in liquid nitrogen, and stored at -80° C until further analysis. Plasma was isolated by centrifugation at 5,000g for 15 min at 4° C and stored at -80° C until analysis.

Triglyceride (TG) and Cholesterol (CH) Assay. TG and CH levels were determined in animal plasma and liver. Samples were combined with Infinity Triglyceride Reagent kit or Infinity Cholesterol Reagent kit (Thermo Electron Corporation, Waltham, MA) following manufacture's instructions. Detection was determined by the absorbance detected at 570nm with the Perkin-Elmer multiplate reader, Wallac Victor2 (Perkin Elmer Life Sciences).

Tissue Lipid Analysis. Frozen liver total lipids were extracted in homogenizing buffer containing (0.3M Sucrose, 25mM 2-mercaptoethanol, 10mM EDTA at pH 7.0) for 30 seconds with an electric homogenizer. The homogenate was combined with 2.5 volumes of chloroform, vortexed and incubated for 30 minutes at room temperature. An additional 2.5 volumes of 1:1 ratio of chloroform: 0.15M NaCl, vortexed and incubated at room temperature for an additional 1h. Homogenate phases were centrifuged at 3000 x g for 10 minutes, and the chloroform layer was dried under vacuum. The lipids were re-suspended in 95% ethanol and followed by triglyceride and cholesterol assay using enzymatic assay kits (Thermo DMA Inc., Louisville, CO).

Protein Determination. The Bradford method was used to determine protein concentrations, using a commercial protein assay kit (BioRad, Hercules CA) according to the Manufacture's protocol and compared to bovine serum albumin as a standard.

Non-esterified Free Fatty Acids (FFA). FFA were measured using NEFA C assay kit from Wako (Richmond VA) according to manufacture's instructions.

Adiponectin. Adiponectin levels in animal plasma was measured with a commercially available Adiponectin microwell ELISA kit (Linco, St. Charles,

MO) according to the manufacture's protocol.

Statistical Analysis. All values are represented as mean \pm SD. Biochemical and molecular analysis were performed in duplicates. Statistical significance was analyzed using Student's t-test. P-values \leq 0.05 were considered statistically significant.

Results

The recommended dosage of noni juice varies depending on the severity of the condition that the consumer wishes to alleviate. Therefore, we decided to provide 1.5 μ l of noni juice per gram of body weight, twice daily, which is equivalent to the highest recommendation of 3oz two times a day for a sick person wishing to diminish the ailment (<http://nonipower.blogspot.com/2006/04/noni-dosage.html>, <http://www.noni-is-good-for-you.com/>).

Effects of Noni Juice on Animal Body Weight, Food Intake, and Adipose Weight. Animal body weight and feed intake was monitored daily throughout the course of the treatment. Figure 2.1 shows the daily averages of the body weight Con, Con + NJ, HFD, and HFD + NJ. At the end of the 5 weeks, Con + NJ had significantly reduced body weight gain (60% of control) when compared to control. Whereas the HFD had gained significantly more weight (115%) compared to control. The HFD + NJ mice gained less weight than untreated control, though it was not significant, However, the weight gained by HFD + NJ was 35% less than the untreated HFD group (Table 2.1, $p < 0.05$).

When the food intake was converted to kcal consumed, the Con + NJ mice consumed a significant 8% less kcal compared to control. The HFD group consumed 10% more kcal compared to control, whereas HFD +NJ was not

statistically different compared to the untreated control group. HFD + NJ did consume significantly less kcal compared to the high fat diet alone (Table 2.1, $p < 0.05$).

At sacrifice, there were no observed differences in wet adipose tissue weight between untreated and treated mice consuming a control diet. However, the wet adipose tissue weight of the HFD group was significantly heavier (55%) compared to Con. HFD + NJ also did not exhibit any statistically significant difference in adipose tissue weight compared to the untreated Con or HFD (Table 2.1, $p < 0.05$).

Effects of Noni Juice on Fasting Blood Glucose Levels. The midpoint and endpoint plasma glucose was measured after a 4 hour fast. There were no significant changes between all groups. However the mice consuming a HFD did have slightly elevated blood glucose levels compared to the untreated control. The glucose levels between the HFD and HFD + NJ were not statistically different (Table 2.1).

Glucose Tolerance Test and Insulin Tolerance Test. Figure 2.2a shows the GTT from time 0 to 120 minutes after i.p. injection with dextrose solution. After 30, 60, and 120 minutes from the injection time Con + NJ showed no significant differences compared to Con. The HFD group on the other hand showed no

significant differences at 30 minutes, but did show significantly elevated blood glucose levels at 60 and 120 minutes (40% and 25% respectively higher, $p < 0.05$). Through out the GTT, HFD + NJ mice showed no significant differences compared to the untreated control, but they did have significantly reduced glucose levels at 60 and 120 minutes when compared to HFD alone. The ITT was also conducted after a 4h fast. At 30, 60, and 120 minutes the Con + NJ showed no statistically significant variation compared to Con, the HFD group showed the same results as Con + NJ when compared to Con. However, the HFD + NJ showed some variance when compared to the untreated control group. At 30 and 60 minutes, the disparity was not statistically significant, but at 120 minutes the blood glucose levels were significantly lower ($p < 0.05$). In spite of this, there were no significant difference when HFD + NJ was compared to HFD alone.

Effects of Noni Juice on Plasma and Hepatic Triglyceride and Cholesterol.

After 5 weeks of treatment the mice were sacrificed and the plasma and hepatic lipids were analyzed. The plasma triglyceride levels were not significantly lower in Con + NJ compared to control, however it was reduced by 15%. Though there was a slight reduction in the plasma triglyceride in Con + NJ, the same can not be said for HFD and HFD + NJ which were significantly greater than control ($p < 0.05$). Never the less, the noni treated HFD group did have a 13% reduction compared to the HFD group not treated with noni. The plasma CH levels were

also measured. Although Con + NJ had a 10% decrease in plasma CH compared to control it was no significant. The HFD and HFD + NJ groups did have a significant 30% elevation in plasma CH when compared to Con. There were however no differences between the treated and untreated high fat diet groups (Figure 2.3b, $p < 0.05$).

Figure 3.4a shows TG measured from lipids extracted from the liver tissue. Hepatic triglycerides were measured in each of the groups. The Con + NJ had a significant 40% increase in hepatic TG when compared to Con. The HFD and HFD + NJ also showed significant increases in hepatic TG (259% and 249% of untreated control). There was only a 10% decrease in HFD + NJ compared to HFD alone, and this was not significant ($p < 0.05$).

Figure 3.4b shows that there were no observed changes in any of the groups hepatic CH levels when compared to control.

Effects of Noni Juice on Circulating FFA and Adiponectin. The free fatty acids measured in the plasma showed no significant difference between all groups (Figure 3.5a). Figure 3.5b shows the adiponectin released from the adipose into the blood. The Con + NJ groups showed no significant variation compared to untreated control. However, the adiponectin levels were significantly decreased by up to 22% in the HFD and HFD + NJ groups when compared to control ($p < 0.05$). There was no significant variation between HFD and HFD + NJ.

TABLE 2.1

Effects of noni juice consumption on body weight, food intake, and adipose tissue weight in C57BL/6J Mice

| | Control Diet | | High Fat Diet | |
|---------------------------|--------------|---------------------------|--------------------------|--------------------------|
| | | Noni Juice | | Noni Juice |
| Body Weight Gained (g) | 5.78 ± 1.50 | 3.42 ± 0.53 ^a | 6.66 ± 1.19 ^b | 4.36 ± 1.06 ^c |
| Diet Consumed (g/day) | 2.80 ± 0.11 | 2.58 ± 0.16 ^a | 2.25 ± 0.10 ^a | 2.04 ± 0.08 ^a |
| Diet Consumed (kcal/day) | 11.38 ± 0.44 | 10.48 ± 0.65 ^a | 12.5 ± 0.54 ^b | 11.35 ± 0.45 |
| Adipose Tissue Weight (g) | 1.15 ± 0.35 | 0.99 ± 0.26 | 1.78 ± 0.25 ^b | 1.43 ± 0.32 |
| Midpoint Glucose (mg/dl) | 156.4 ± 25.5 | 135.2 ± 16.8 | 159.6 ± 22.9 | 152.0 ± 9.41 |
| Endpoint Glucose (mg/dl) | 176.4 ± 43.4 | 155.2 ± 22.8 | 188.4 ± 20.5 | 167.0 ± 7.31 |

Data are represented as mean values ± SD (n=5)

a = significant reduction compared to Con

b = significant increase compared to Con

c = significant decrease compared to HFD

Significance = p ≤ 0.05

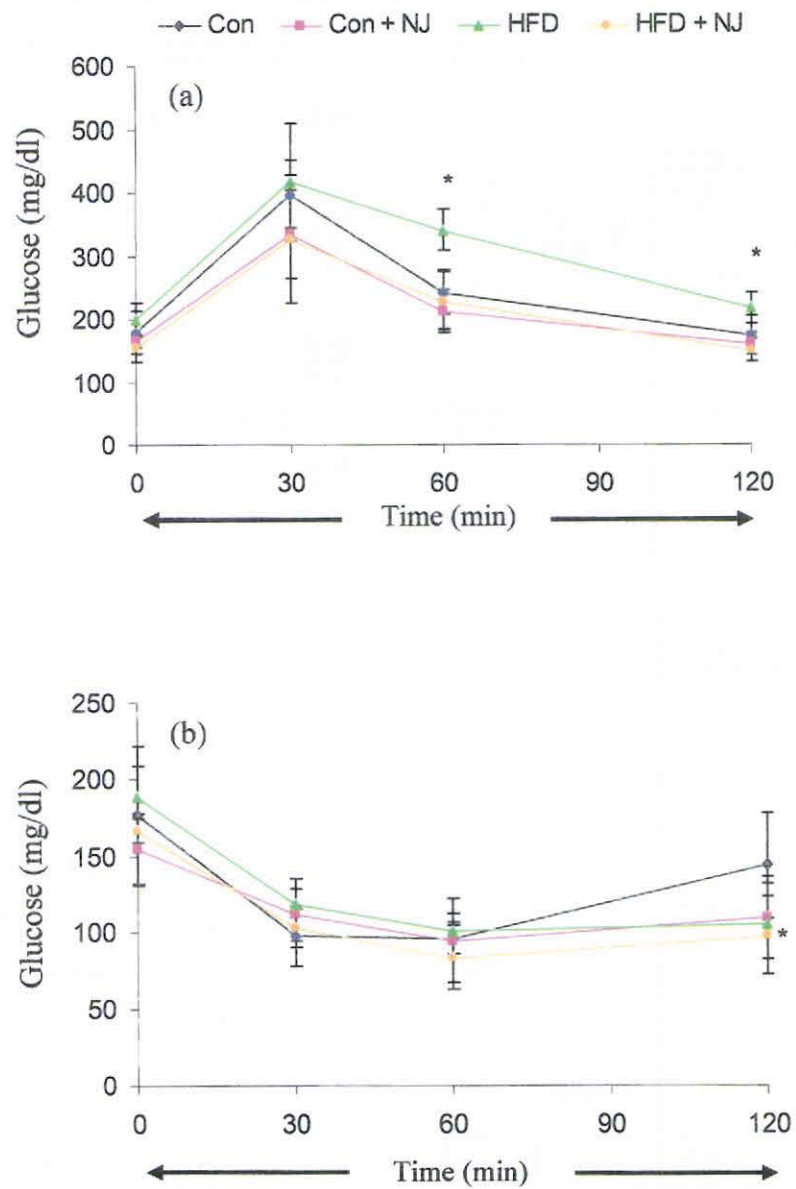


Figure 2.2: Effects of noni juice consumption on glucose tolerance test (a) and insulin tolerance test (b) Data are represented as mean values \pm SD (n=4), $p < 0.05$.

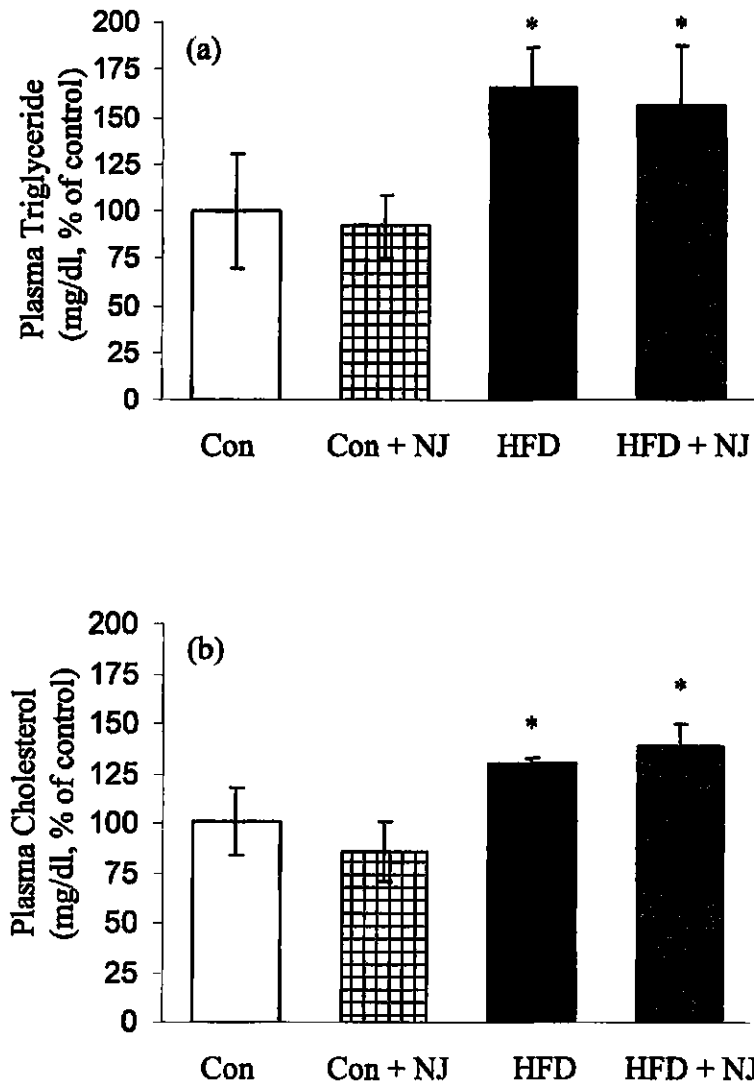


Figure 2.3: Effects of noni juice consumption on plasma triglyceride (a) and cholesterol levels (b), in C57BL/6J mice. Data are represented as a percentage of the control (set as 100%). Values are \pm SD (n=4), $p \leq 0.05$.

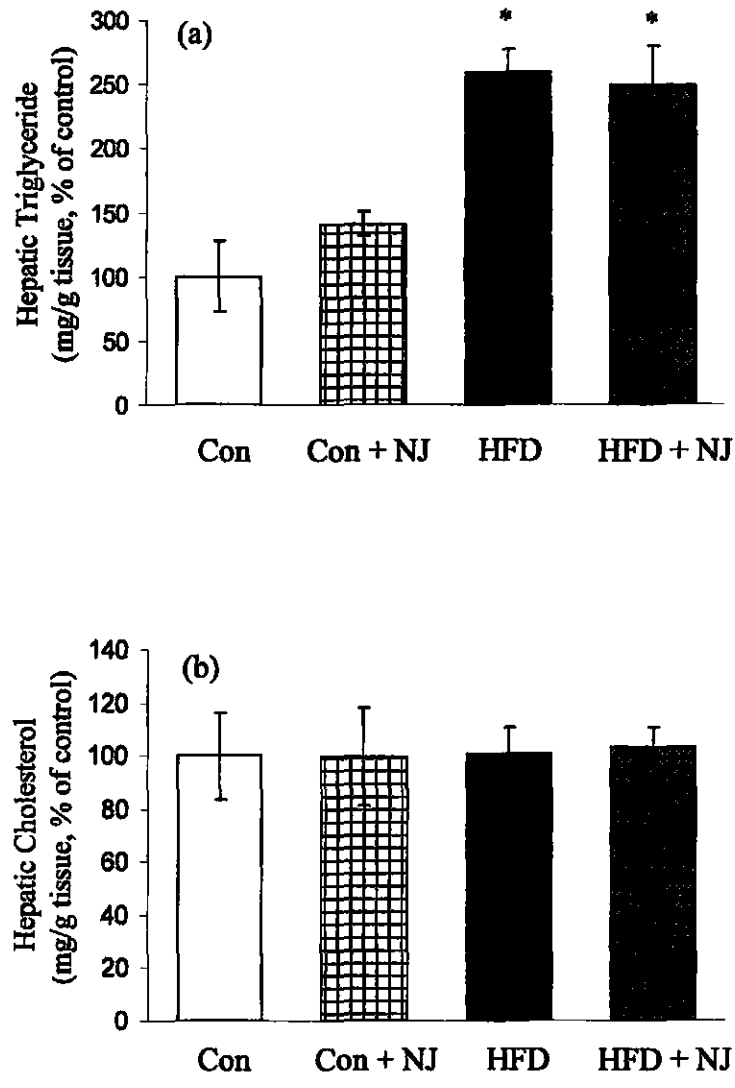


Figure 2.4: Effects of noni juice consumption on hepatic triglyceride (a) and cholesterol (b) levels in C57BL/6J mice. Data are represented as a percentage of the control (set as 100%). Values are \pm SD (n=4), $p \leq 0.05$.

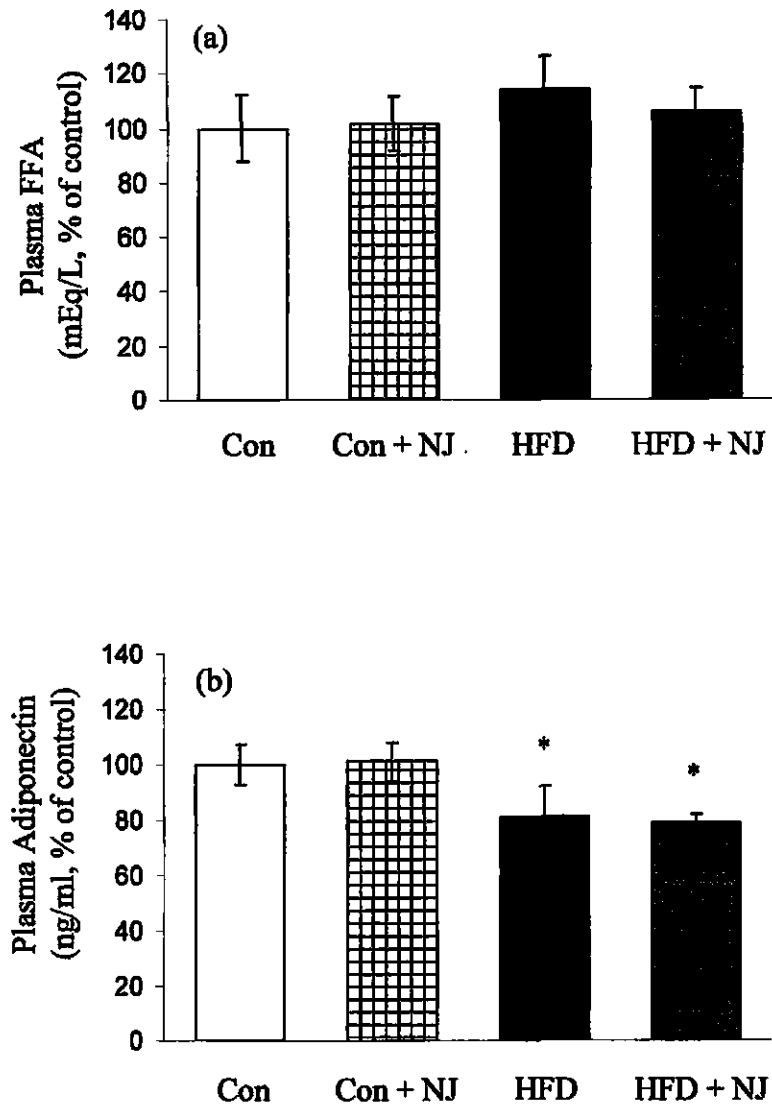


Figure 2.5: Effect of noni juice consumption on plasma free fatty acid (a) and plasma adiponectin (b) in C57BL/6J mice. Data are represented as a percentage of the control (set at 100%). Values are means \pm SD (n=4), $p \leq 0.05$.

DISCUSSION

In this study, we examined the effects of noni juice as a health supplement in diet induced obese mice over a five week period. Our results showed that body weight gained in the groups supplemented with noni juice was significantly reduced when compared to controls; this effect was more marked in animals fed a control diet with noni juice. Along with the decrease in body weight gained, the adipose tissue weight was significantly reduced. There was however a slight decrease in the energy intake of the C57BL/6J mice in both control and HFD treated mice, which may be attributed to some of the weight loss

Noni juice also improved glucose tolerance, which develops in high-fat diet fed C57BL/6J mice. There was no significant difference in treated mice compared to control, whereas a significant increase in blood glucose levels at 60 and 120 minutes was observed in mice fed a high fat diet alone. Nayak et al. (2007) have previously shown that fasting glucose levels in the diabetic induced noni treated experimental animals was reduced by 29% compared with diabetic experimental control animals. The fasting blood glucose levels were slightly reduced in the noni treated mice, however it was not significant. The difference in our study and that of Nayak et al. (2007) may be due to the fact that the animals in their study were diabetic rats, whereas we used a diet induced obese mouse model.

Evidence is presented to show that, in addition to the hypoglycemic activity of noni juice, it also possess lipid lowering properties. A major risk

factor for atherosclerosis is elevated blood lipid levels (Castelli *et al.*, 1986). Over the past few years, the hunt for new drugs with the ability to reduce and/or regulate serum cholesterol and triglycerides levels, lead to a handful of reports on significant actions of natural agents (Jahromi & Ray, 1993). In light of previous evidence that many plants are successful in the prevention of hyperlipidemia, evaluation of the effects of noni juice on lipid regulation in diet induced obese C57BL/6J mice was conducted. Our study shows that plasma and hepatic lipid levels were moderately regulated by treatment with noni juice. In plasma the triglyceride and cholesterol levels were slightly reduced at the end of the four week study, however it was not able to induce significant changes. From this study, there is slight evidence that noni juice may possess the ability to partially reverse the hyperlipidemia in diet induced obese mice. It may be possible that the use of noni juice extracts may have more profound effects.

Although the increases in triglycerides and cholesterol levels are a major component in the risk for metabolic abnormalities, it has also been shown that free fatty acids and adiponectin play a large role. In our study, we examined the effects of noni treatment on FFAs and adiponectin. However, we observed non-significant changes in free fatty acids in all groups compared to control. Adiponectin is usually found inversely proportional to body weight. Consistent with this, we found the levels of adiponectin in the circulating plasma were significantly reduced by 20% and 22% in the HFD and HFD + NJ groups.

However, we would expect to see a slight increase in adiponectin in the noni treated groups.

In summary, noni juice did have some hypoglycemic properties, and may have some hypolipidemic properties. However, it did not have any beneficial effects regulating the adipocytokines or FFA. Further studies to elucidate the mechanisms of action are warranted and characterization of active components of noni, such as phenolics or related analogues may help to elucidate the specific actions of noni.

CHAPTER 3
LIPID PROFILES OF HEPG2 AND 3T3-L1 CELL CULTURE
TREATED WITH NONI JUICE

Introduction

Human hepatoma cells, HepG2 are commonly used as an *in vitro* model to examine hepatic lipoprotein metabolism. The transport of triglyceride, cholesterol, and lipids from the liver to other tissues occur through the formation of lipid and apolipoprotein containing lipoproteins. Lipoproteins are classified into several groups based on the lipid content and density and are synthesized and secreted from HepG2. The most important regulatory substrate for the assembly and secretion of hepatic apoB lipoproteins are fatty acids (Fielding *et al.*, 1995, Julius, 2003). The delivery of fatty acids to the liver is often via albumin, which provides a stimulus for the hepatic assembly and secretion of apoB (Zhang *et al.*, 2004). Therefore, we chose HepG2 cells to examine the *in vitro* effects of noni juice on hepatic lipid metabolism and secretion. Adipocytes are also an important site for the storage and accumulation of triglyceride and the release of glycerol and free fatty acids. Pre-adipocyte cells lines are also useful for the study of morphological changes and lipid accumulation in adipose tissue. Therefore, we used the 3T3-L1 pre-adipocyte cell line to study the *in vitro* effects of noni on cell differentiation, lipid accumulation, and glycerol release.

Material and Methods

HepG2 Cell Treatments. HepG2 cell line was obtained from American Type Culture Collection. The cells were cultured using T75 flasks (Corning Incorporated, Corning NY) in minimum essential medium eagle (MEME) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin in 5% CO₂ at 37°C. Cells were split when they reached 85% confluence and used until passage 12. Cells were trypsinized and seeded in clear 24-well and 96-well culture plates (Corning Incorporated, Corning, NY). Cells were grown to 80-90% confluency, then incubated for 24h in serum-free media containing 1% bovine serum albumin (BSA). After 24 h, cells were further incubated with either the control medium (MEME with 1% BSA) or treated with commercial noni juice at concentrations of 1.5 and 3% (v/v) in the control medium with 0.8mM oleate. Chemicals were obtained from Sigma, St. Louis, MO.

Cellular TG and CH. To measure cellular total TG and cholesterol levels, HepG2 cells were washed with PBS and lysed with 0.5N sodium hydroxide (NaOH). TG and CH levels were measured using the infinity TG reagent and infinity CH reagent previously mentioned. Total TG and CH levels were normalized to mg of protein as determined by Bradford assay (Bio-Rad laboratories, Hercules, CA, USA).

Apolipoprotein ELISA. Commercially available apoB microwell ELISA assay kit (AlerCHEK, Inc. Portland, ME) was used to determine apoB levels secreted into the culture media according to the manufacturer's instructions.

3T3-L1 Cell Treatments. Murine 3T3-L1 preadipocytes were obtained from American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured according to manufacturer's instructions. Pre-adipocytes were cultured in DMEM, containing 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in 5% CO₂ at 37°C. 2 days post confluence, adipocyte differentiation was induced with a hormonal mix in Dulbecco's minimum essential medium (DMEM) containing containing 10% FBS, 167mM insulin, 0.25mM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (Day 0). The cells were incubated for 36 h in the hormonal mix at a constant of 5% CO₂ at 37°C. Cells were then fed and insulin mix in DMEM supplemented with 10% FBS and 167mM insulin on days 3 and 5, after which they were fed on every odd day with DMEM containing 10% FBS. Chemicals were obtained from Sigma, St. Louis, MO.\

Oil Red O Staining. Oil red O staining was used during and adipocyte differentiation to monitor intracellular lipid accumulation. 3T3-L1 cells were washed with cold phosphate buffered saline (PBS) and fixed in 10% formalin overnight. A stock solution of Oil Red O solution was prepared with (0.5 g in 100

ml isopropanol), and passed through a No. 1 filter paper. 6 ml of this solution was mixed with 4 ml of distilled water making a 60% working solution. The solution was incubated at room temperature for 30 minutes and passed through a No. 1 filter prior to use. Chemicals were obtained from Sigma, St. Louis, MO.

Lipolysis Assay. The culture media of cells that had been treated during and after adipogenesis were collected and frozen at -20°C until analysis. The concentration of glycerol in the medium was measured with free glycerol assay reagent (Sigma, St Louis, MO), according to the manufacturer's instructions. The concentrations of glycerol in the media were corrected to a glycerol solution.

Statistical Analysis. All values are represented as mean \pm SD. Biochemical and molecular analysis were performed in duplicates. Statistical significance was analyzed using Student's t-test. P-values ≤ 0.05 were considered statistically significant.

Results

ApoB Secretion from HepG2 Cells Treated with Noni Juice. Noni treatment significantly decreased apoB secretion by 22% and 27% in HepG2 cells treated with 3% noni (v/v) for 24 and 48h under basal conditions when compared to control ($p < 0.05$). HepG2 cells were treated with oleic acid to stimulate lipid synthesis and increase apoB secretion. ApoB secretion was significantly increased in all cells treated with oleic acid compared to untreated control, however treatment with noni did not significantly reduce secretion.

HepG2 Cellular Triglyceride Mass in Cells Treated with Noni Juice. Figure 3.2 demonstrated that under basal conditions treatment with 1% noni (v/v) for 24h significantly reduced cellular triglyceride by 18%, however 3% noni (v/v) was only moderately reduced ($p < 0.05$). On the other hand, in the presence of excess lipids, untreated and treated cells significantly increased cellular triglyceride mass by approximately 2000%. After 48h of treatment both 1% and 3% noni (v/v) significantly reduced cellular triglyceride mass by 20% and 24% respectively, but increased approximately 1600% under lipid rich conditions ($p < 0.05$).

HepG2 Cellular Cholesterol Mass in Cells Treated with Noni Juice. HepG2 cells treated with 1% noni for 24h significantly reduced cellular cholesterol by 16% under basal conditions, but under lipid rich conditions, 1% and 3% noni

increased cellular cholesterol by 17% and 58% respectively ($p < 0.05$). 48h of noni treatment failed to induce changes in cholesterol levels under basal conditions.

Lipid Accumulation in 3T3-L1 Cells Treated with Noni Juice. To assess the effect of noni on lipid accumulation in mature adipocytes, we measured the triglyceride content of differentiated 3T3-L1 adipocytes. Visual inspection by microscopy and Oil red O displayed no differences in morphology of lipid content when treated with noni after adipogenesis. Although there were no visual changes present, the triglyceride mass was significantly decreased in cells treated with 1% and 2% noni, but not in 3% (v/v) treated up to 48h. 3T3-L1 cells treated with noni during differentiation visually displayed through microscopy and Oil Red O staining an increase in lipid droplet accumulation. Treatment with noni during adipogenesis significantly increased triglyceride mass compared to control. At day 5 of the differentiation, triglyceride mass exhibited a 239%, 221% and 161% increase compared to control cells (1%, 2% and 3% respectively). 1% noni treatment during differentiation at day 7, 9 and 11 seemed to plateau at about 170 – 180%, 2% noni treatment plateaued at 150 – 160%, whereas 3% noni decreased to 125% of control at day 9, but rose back to 167% at day 11.

Glycerol Release from 3T3-L1 Cells Treated with Noni Juice. Glycerol release was measured in the media from the 3T3-L1 cells treated during and after

adipogenesis. Both during and after had significantly increased glycerol release from 3T3 cells. At 1%, 2% and 3% noni (v/v) there were significant increases of 23%, 25%, and 49% respectively. As with treatment after adipogenesis, cells treated during adipogenesis showed a marked increase in glycerol release. Treatment with noni at 1% (v/v) during adipogenesis increased glycerol release at day 5, 7, 9 and 11. However significance was only reached at day 9 and 11 increasing lipolysis by 45%. 2% noni (v/v) significantly increased free glycerol in the media by up to 73% of control at day 9 and 63% at day 11. The most profound effects were observed in adipocytes treated with noni during differentiation with 3% noni (v/v). Treatment with 3% noni juice significantly increased glycerol release through out the differentiation process by up to 207% at day 9.

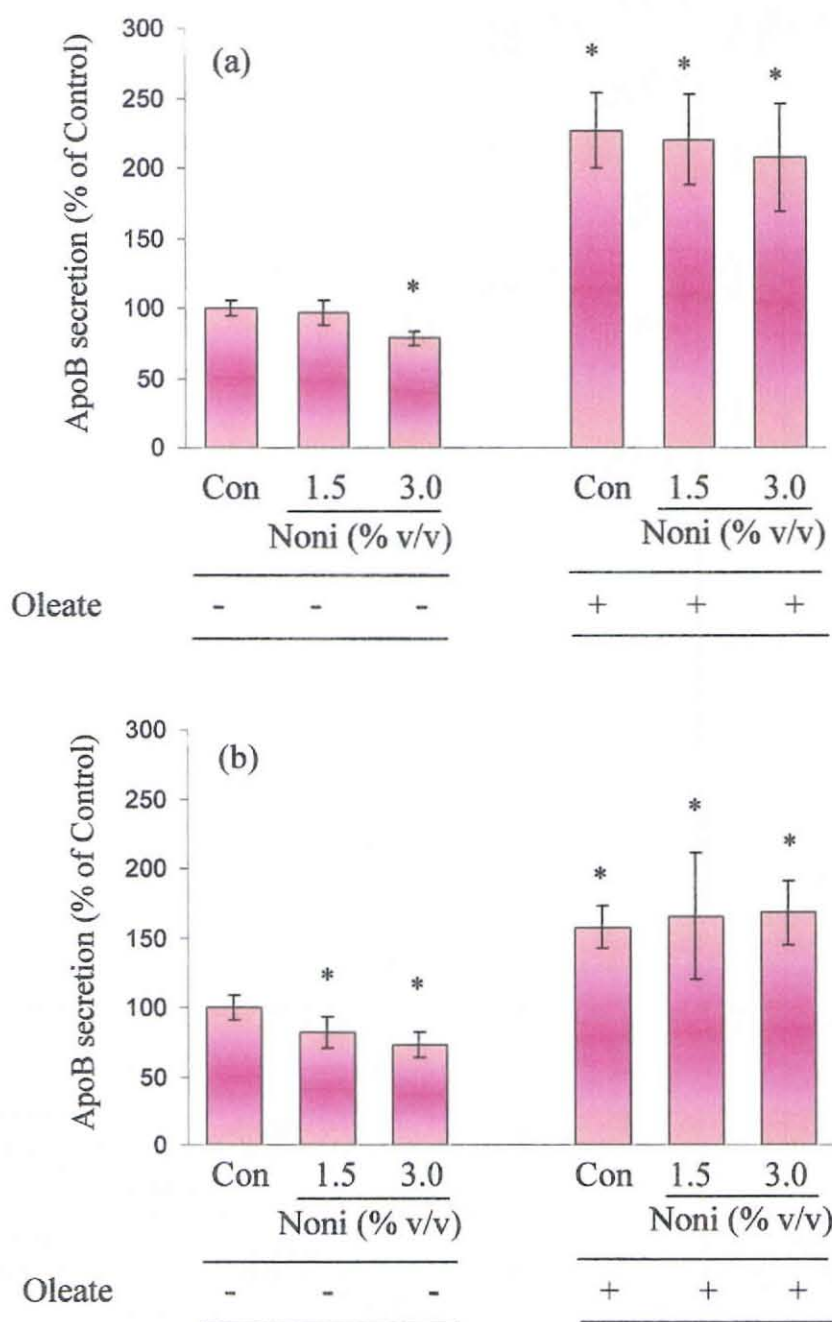


Figure 3.1: ApoB secretion from HepG2 cells. Cells were treated with 1.5 and 3.0% (v/v) noni juice for 24 (a) and 48 h (b) in the presence of absence of oleate. Data are represented as a percentage of the control (set as 100%). Values are means \pm SD (n=6), $p < 0.05$

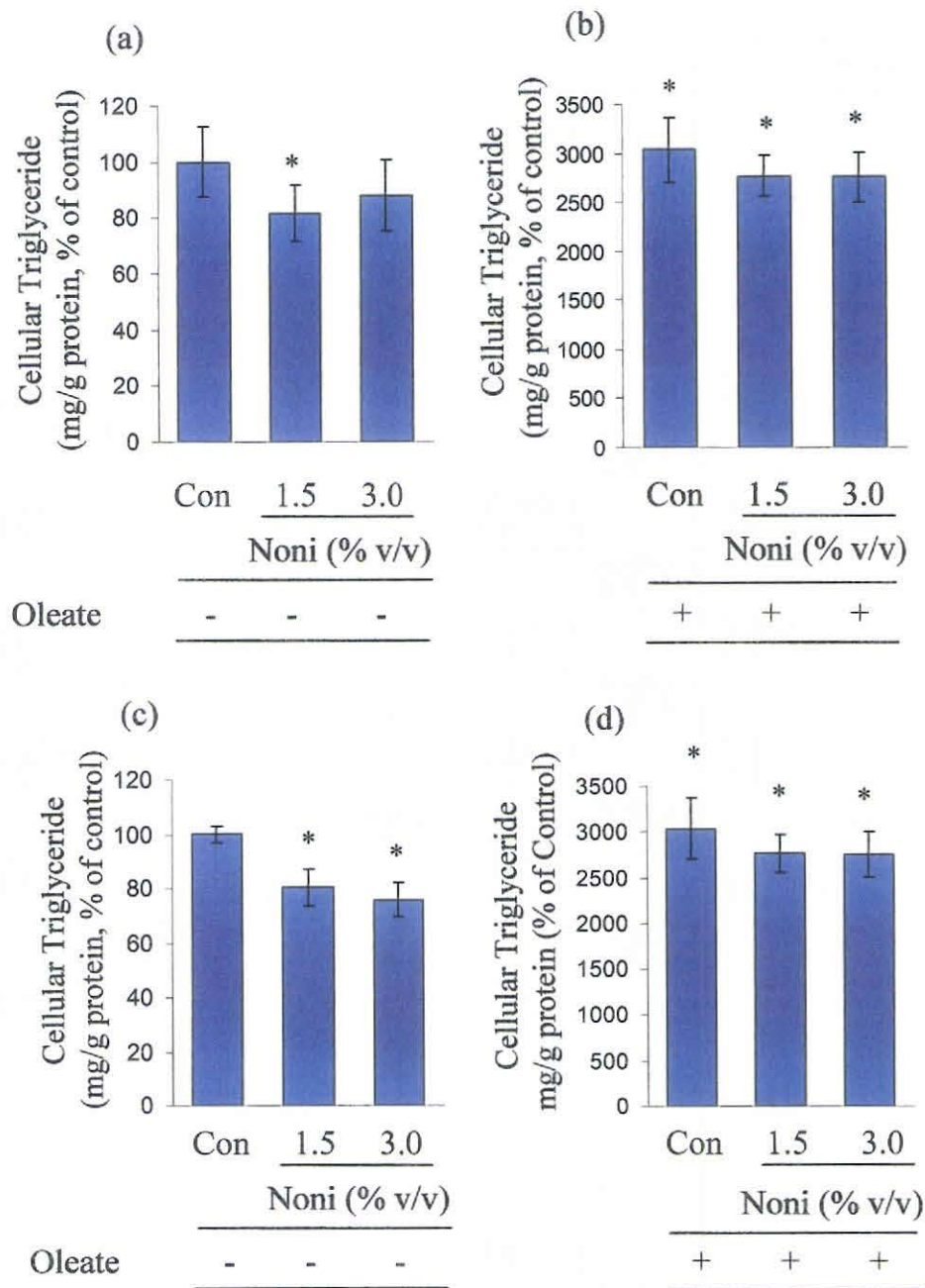


Figure 3.2: Synthesis of cellular TG in HepG2 cells. Cells were treated with noni juice for 24 (a,b) and 48 h (c,d), in the presence or absence of oleate. Data are represented as a percentage of the control (set as 100%). Values are means \pm SD (n=6), $p \leq 0.05$

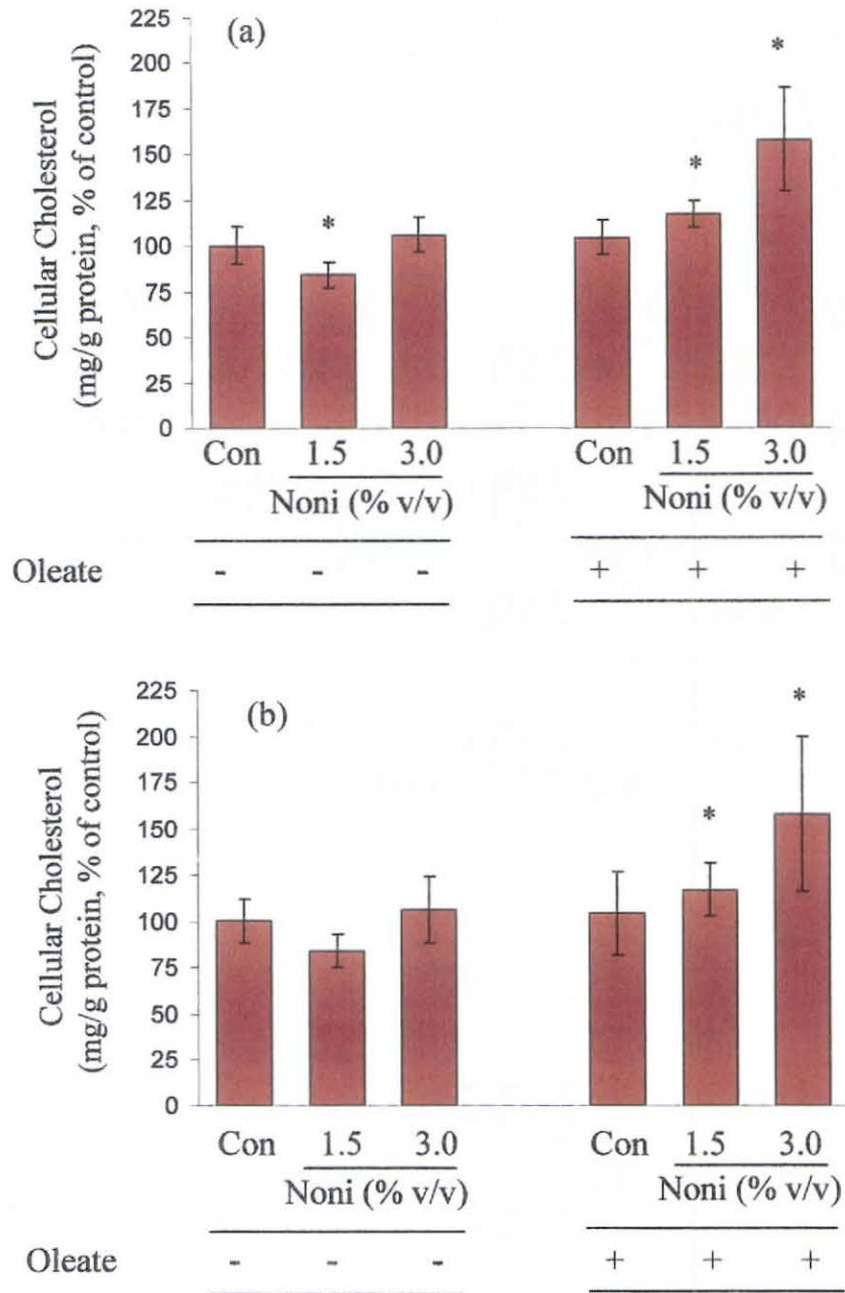


Figure 3.3: Synthesis of cellular CH in HepG2 cells. Cells were treated with noni juice for 24 (a) and 48 h (b), in the presence or absence of oleate. Data are represented as a percentage of the control (set as 100%). Values are means \pm SD (n=6), $p < 0.05$

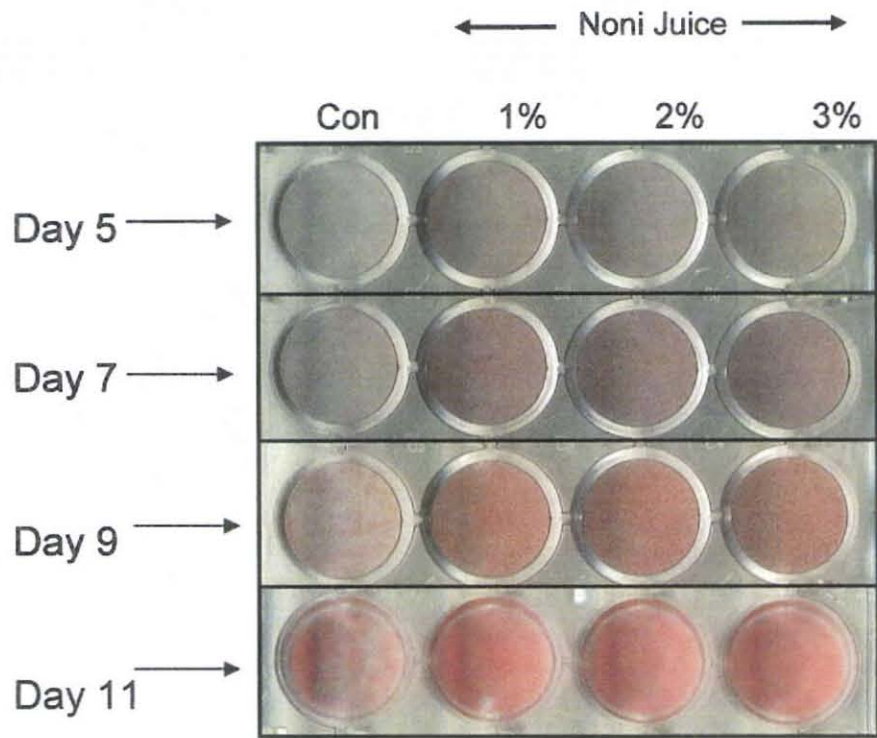


Figure 3.4: Oil Red O Staining of lipid accumulation in 3T3-L1 cells. Murine 3T3-L1 cells were treated with 1, 2, and 3% (v/v) noni juice during differentiation.

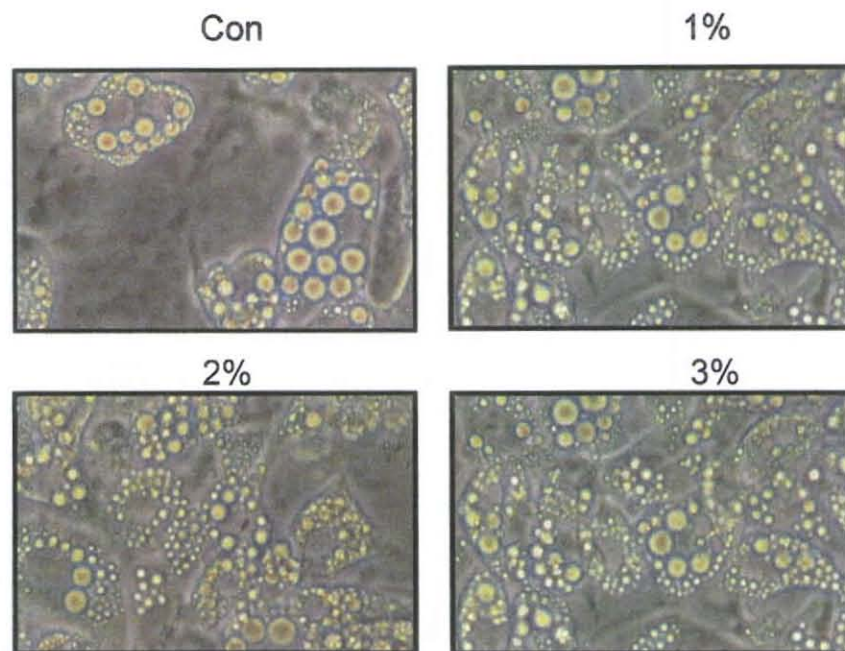


Figure 3.5: Photograph (40x magnification) of lipid accumulation in 3T3-L1 cells. Murine 3T3-L1 cells treated with 1, 2, and 3% (v/v) noni juice during differentiation at day 11.

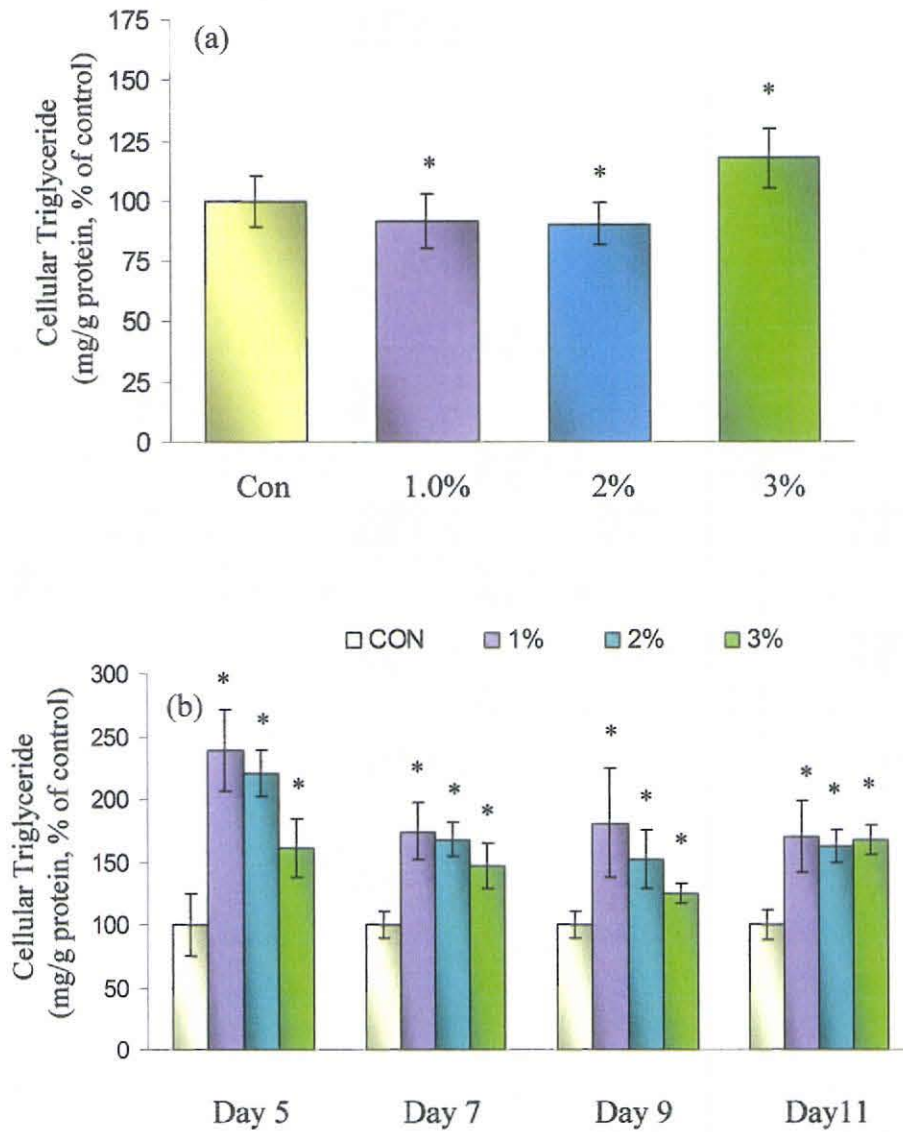


Figure 3.6: Cellular triglyceride mass in 3T3-L1 cells. Cells were treated with 1, 2, and 3% (v/v) noni juice 48h after adipogenesis (a) and during adipogenesis (b) for 5, 7, 9, and 11 days, respectively. Data are represented as a percentage of the control (set at 100%). Values are means \pm SD (n=9 and n=5, respectively), $p \leq 0.05$.

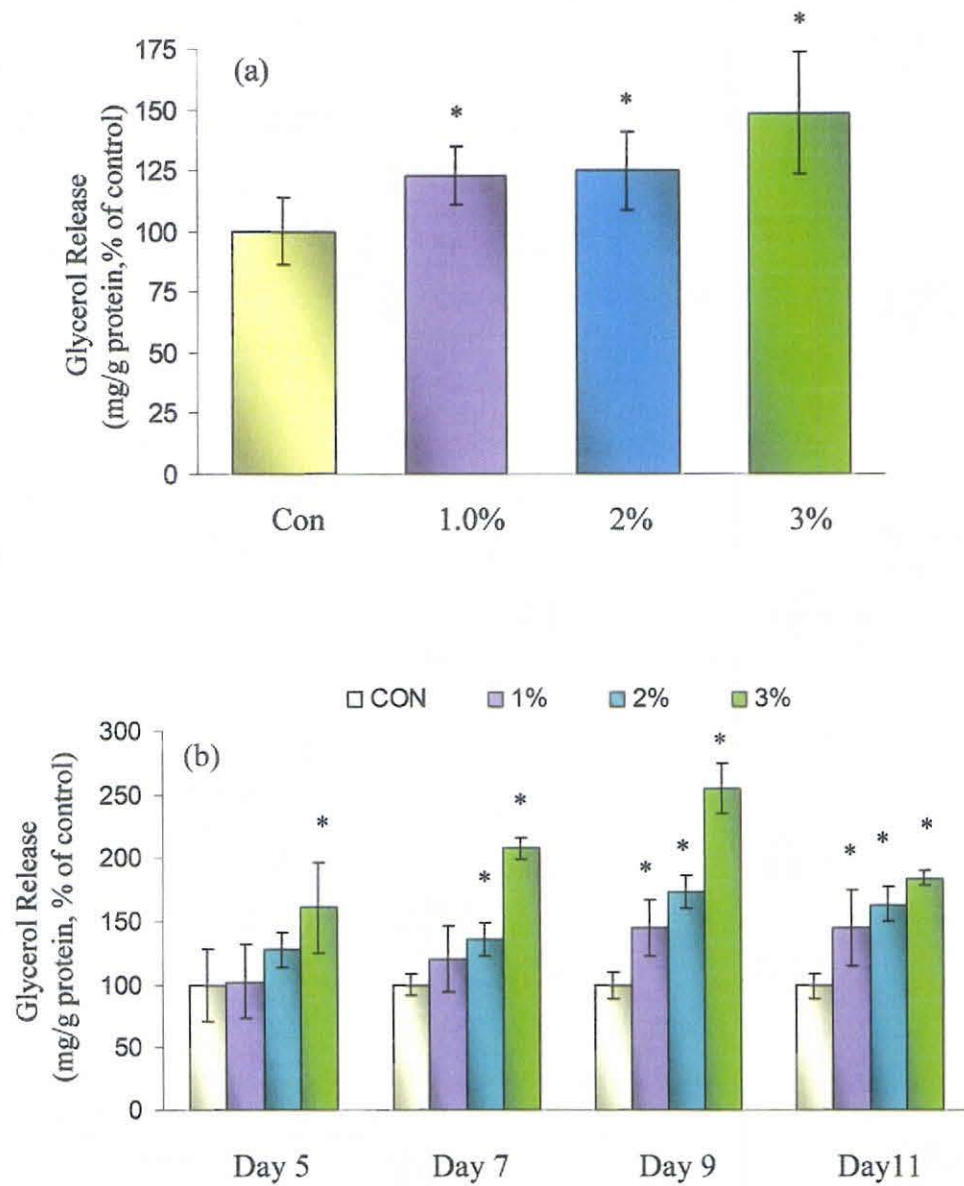


Figure 3.7: Glycerol release from 3T3-L1 cells. Cells were treated with 1, 2, and 3% (v/v) noni juice 48h after adipogenesis (a) and during adipogenesis (b) for 5, 7, 9, and 11 days, respectively. Data are represented as a percentage of the control (set at 100%). Values are means \pm SD (n=9 and n=5, respectively), $p \leq 0.05$.

DISCUSSION

Examining hepatic lipoprotein metabolism utilizing HepG2 is a common practice. The overproduction of ApoB containing lipoproteins has been recognized as a factor responsible for hyperlipidemia (Arad *et al.*, 1990, Vega *et al.*, 1991) and has therefore become an active area of investigation.

Hepatocytes mainly synthesize and secrete apoB as VLDL before it is converted to LDL in circulation. ApoB containing lipoprotein particles in hepatocytes is a complex process that involves the synchronized synthesis and assembly of apoB, cholesterol esters, triglyceride, phospholipids, as well as other components (Boren *et al.*, 1994). Translocation of the newly synthesized apoB across the endoplasmic reticulum allows for the associations with lipid components and assembly of apoB lipoproteins (Sparks & Sparks, 1985). The most important regulatory substrate for the assembly and secretion of hepatic apoB lipoproteins are fatty acids (Fielding *et al.*, 1995, Julius, 2003). The delivery of fatty acids to the liver is often via albumin, which provides a stimulus for the hepatic assembly and secretion of apoB (Zhang *et al.*, 2004).

In our study, we found that in the absence of excess lipids, apoB secretion was significantly reduced up to 27% when treated with 3% noni juice (v/v). However, the same was not observed when cells were incubated lipid bioavailable media in the form of oleic acid. Under these conditions all cells produced a dramatic increase in apoB secreted into the media compared to untreated

control. The decrease observed in the cells treated with noni was not significant when compared to control plus oleic acid.

Cellular triglyceride mass plays a vital role in the formation of apolipoproteins. Increases in triglyceride synthesis will also increase apoB lipidation and facilitate secretion, consequently, degradation of the apoB is prevented. It is therefore important to consider the effects of triglyceride mass on apoB secretion. When cellular triglyceride was measured in the HepG2 cultures, we saw a significant decrease in cells treated with noni under basal conditions. Consistent with the secretion of apoB previously mentioned, we found that there were no significant reductions in cellular triglyceride mass when oleic acid was present in the media. ApoB secretion slows in the absence of adequate lipid availability and allows for degradation, therefore, you can see a direct correlation between triglyceride mass and reduced apoB secretion when HepG2 cells are treated with noni. However, in the excess of lipids, there was also an increase in triglyceride, allowing for the increased secretion of apoB. The presence of excess lipid and noni treatment there was an increase in cellular triglyceride, allowing for the constitutively expressed apoB to increase secretion due to an increase in translocation and reduced degradation through proteosomal pathways (Dixon & Ginsberg, 1993). The role of cholesterol in apoB synthesis and secretion is less well established. There have been conflicting reports on the role of cholesterol or cholesteryl ester availability in regulating hepatic apoB secretion. Studies by Fuki

et al. (1989) showed that the direct addition of cholesterol to the medium of HepG2 cells increased the secretion of apoB. However, Dashti (1992) and colleagues showed that the secretion of apoB was unaffected by the addition of free cholesterol to the medium of HepG2 cells. In our study, we found that under basal conditions, cholesterol was significantly reduced. However, when excess lipids were in the medium, there was a significant increase in cellular cholesterol when treated with noni. Although cholesterol was significantly increased, apoB secretion did not show the same trend.

Adipose tissue is very important in the maintenance of glucose, lipid, and energy homeostasis. One of its major functions is the uptake of glucose and free fatty acids, followed by storage as triglyceride. It is also responsible for supplementing FFA and glycerol by lipolysis of the stored triglyceride to other tissues.

We showed after 3T3-L1 cells had undergone differentiation into mature adipocytes treatment with noni for 48h significantly decreased cellular triglyceride levels at 1% and 2% but not 3%. When the cells were treated with noni during the differentiation process, there were significant increases in all treatment groups. The decrease in cellular triglyceride may be a result of increased lipolysis, which is the brake down of triglycerides into glycerol and fatty acids. Once broken down, the free fatty acids and glycerol are released into the bloodstream where hydrophobic free fatty acids can bind to serum albumin for transport to tissues and

glycerol can be absorbed by the liver or kidney. The glycerol released from 3T3-L1 cells were significantly increased in the media when treated both during and after differentiation. From the results in our study, one possible reason for the decrease in cellular triglyceride when treated after differentiation is due to the increased hydrolysis and release of glycerol and free fatty acids into the media. However, when cells were treated during differentiation there was an increase in cellular triglyceride as well as glycerol in the media. The rate of increase in differentiation and lipid formation may have been too great for lipolysis to regulate the decrease in cellular triglyceride, though there was a significant increase in glycerol in media.

CHAPTER 4
LONG TERM STUDY:
HEALTH EFFECTS OF NONI JUICE ON C57BL/6J MICE

Introduction

The lifestyle of the 21st century, with the increase in caloric intake and lack of physical activity has sharply increased the incidence of obesity and predisposes the population to obesity related disorders such as dyslipidemia and diabetes. The use of the high fat diet fed C57BL/6J mouse model, originally introduced by Surwit et al. (1988) has been shown to be an effective model for studying obesity and diabetes. Typically, male C57BL/6J mice are fed a high fat diet for 12 weeks. Within a short time, they become obese, slightly hyperglycemic, and develop impaired glucose tolerance which makes them useful for the study of the physiological mechanism of obesity and diabetes.

In obesity and dyslipidemia, there is abnormal regulation of lipids and an increase in triglyceride, cholesterol, and lipoproteins circulating in the blood and stored in tissues. The adipose tissue accumulates excess triglycerides until its subsequent release as non-esterified fatty acids. The excess FFAs are released into the circulation and can accumulate in tissues such as the liver, which ultimately contributes to dyslipidemia and diabetes (Bays *et al.*, 2004). The adipose tissue also secretes adipocytokines, such as leptin, resistin, and adiponectin which are

important factors in insulin dependent glucose uptake. In obese states, these adipocytokines are not regulated properly and add to the increased risk of T2D (Lehmann *et al.*, 1995, Yki-Jarvinen, 2004). These adipocytokines have been shown to be regulated by PPAR γ , which is a nuclear transcription factor important in adipose differentiation. In obese states, PPAR γ is more highly expressed in adipose tissue (Tamori *et al.*, 2002).

The investigations from the 5 week study and cell culture experiments have not been able to produce conclusive results on the health benefits of consuming noni juice. Studies duplicating the first animal experiment are needed to conclusively delineate the effects of noni juice in the animal model. Most studies utilizing a high fat diet are conducted for 12 weeks, thus in the next experiment treatment with noni on a high fat diet for a longer period of time might be able to produce more profound effects. Investigating the effects of noni on different molecules related to obesity, CVD, and T2D would also be useful.

The aim of this study was to investigate the health effects of consuming noni juice over an extended period of time. Also to determine if the regulation of glucose and lipids *in vivo* using C57BL/6J male mouse are dependent on the source of noni juice, be it commercial or homemade juices.

Materials and Methods

Animal Experiments. The experiments were conducted according to the Guidelines for Institute of Animal Care and Use Committee at the University of Hawaii at Manoa. 8 week old male C57BL/6J mice (Jackson Laboratory, Wilmington, MA), weighing 22.5 ± 1.47 g were housed individually. The mice were maintained in a temperature controlled room at 20°C to 22°C and kept on a 12h light/12h dark cycle. Food and water were available ad libitum.

Mice were divided into 6 groups (n=7), control diet (Con), Con + Commercial Juice (Con + CJ), Con + homemade juice (Con + HJ), high fat diet (HFD), and HFD + CJ, and HFD + HJ. Con mice received chow containing 4 kcal % fat and sucrose while HFD mice received chow containing 58 kcal % fat and sucrose. The noni treated groups received treatment of commercial noni juice or homemade noni juice at human consumption levels of 1.5 μ l/g of body weight twice daily for 12 weeks.

The commercial noni juice was purchased from a local health store. The ripe fruit were harvested, washed, and dried. The fruit are packed into tanks and left outside. The juice slowly seeped from the fruit and fermented for about 6-8 weeks. The juice is drained from the tanks, pasteurized (heated to 180 degrees), and bottled for sale.

The homemade noni juice was made with ripe noni fruits that were collected from a tree in Manoa. The fruits were washed, dried, and stored in

a glass container. The container was left in indirect sunlight for approximately 8 weeks, allowing the fruit juice to seep from the skin. The fruit and fruit juice were strained twice and refrigerated for later use.

Animal Measurements. Body weight and food intake was measured daily. Fasting glucose levels were measured from tail vein bleed of mice fasted for overnight (12h) using one touch ultra meter and glucose strips (LifeScan, Milpitas CA) at 4 weeks and 11 weeks. GTT was measured after a 12h fast from tail vein bleed of mice injected intraperitoneally (i.p.) with 2 mg/g body weight of dextrose solution (Phoenix Pharmaceutical, St. Joseph MO) at 4 weeks and 12 weeks. ITT was measured after a 4h fast from tail vein bleed of mice injected (i.p.) with or 0.75 units/kg body weight of insulin (Novolin R, Novo Nordisk Pharmaceuticals, Princeton NJ) at 4 weeks and 11 weeks.

Animals were fasted overnight with free access to water and were anesthetized with isoflourane. Blood was collected by cardiac puncture under anesthesia at the start of the sacrifice. Tissue was excised, weighed and immediately snap frozen in liquid nitrogen, and stored at -80° C until further analysis.

Plasma was isolated by centrifugation at 5,000g for 15 min at 4° C and stored at -80° C until analysis.

Plasma and Liver Lipids and Plasma Chemistry

Plasma and liver TG and CH, protein estimations, plasma FFA, and plasma adiponectin were measured according to the methods described in Chapter 2 (pages 17 to 18).

Resistin. Resistin levels in animal plasma was measured with a commercially available Resistin microwell ELISA kit (Linco, St. Charles, MO) according to the manufacture's protocol.

Leptin. Leptin levels in animal plasma was measured with a commercially available Resistin microwell ELISA kit (Linco, St. Charles, MO) according to the manufacture's protocol.

Whole Cell Adipose Protein Extraction. Frozen adipose tissue was weighed and extracted in homogenizing buffer containing (20mM HEPES, 250mM sucrose, 4mM EDTA, 1% TritonX 100, and complete protease inhibitor cocktail (Roche Applied Sciences, Indianapolis, IN) for 30 seconds with a electric homogenizer making a 10% homogenate. The homogenate was centrifuged at 12,000 x g at 4°C for 20 minutes. The supernatant was transferred to a new tube and stored at -80°C.

Western Blot Detection of ApoA1, ApoB100, and PPAR γ . Protein concentrations in the plasma and adipose tissue extractions were measured using the Bradford method according to the manufacture's protocol (Bio-Rad Lab. Hercules, CA). Equal amounts of protein from the plasma were separated by SDS-gel and transferred onto nitrocellulose membranes. Membranes were blocked in bovine serum albumin for 2h. Primary antibodies against ApoA1 and ApoB100 (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated overnight. HRP conjugated IgG secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated for 2 h at room temperature and detected with Enhanced Chemiluminescent (ECL) detection reagents (Amersham Biosciences, Piscataway, NJ). Equal amounts of protein from the adipose tissue extraction were separated by SDS-gel and transferred onto nitrocellulose membranes. Membranes were blocked in bovine serum albumin for 2h. Primary antibodies against PPAR γ (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated overnight. HRP conjugated IgG secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated for 2 h at room temperature and detected with Enhanced Chemiluminescent (ECL) detection reagents (Amersham Biosciences, Piscataway, NJ).

Statistical Analysis. All values are represented as mean \pm SD. Biochemical and molecular analysis were performed in duplicates. Statistical significance was analyzed using Student's t-test. P-values \leq 0.05 were considered significant.

Results

Animal Body Weight, Food Intake, and Tissue Weight. Animal body weight was measured daily for 12 weeks. Con + CJ and Con + HJ showed no significant differences in bodyweight at the end of the 12 weeks when compared to untreated control. However, Con + CJ mice gained significantly less weight compared to Con. At sacrifice, mice fed a high fat diet weighed significantly more than the untreated control. Figure 4.1 shows that compared to Con, the body weight of HFD group was significantly higher after 7 weeks, whereas the weight of HFD + CJ and HFD + HJ was not significantly higher until weeks 10 and 11, respectively ($p < 0.05$).

The food intake of the mice treated with noni and consuming a control diet was not statistically different compared to the untreated control group. However, the high fat diet fed mice consumed significantly less kcal of food compared to untreated control (Table 4.1, $p < 0.05$). There were no differences in food intake of HFD + CJ and HFD + HJ when compared to HFD alone. The reduction of kcal consumed in the high fat diet may be due to the loss of control food in the cage.

There were no differences in adipose tissue weight of untreated and treated mice on a control diet. There was a significant 3.5 fold increase in adipose tissue weight of high fat diet fed mice when compared to Con (Table 4.1, $p < 0.05$). Noni treated mice on a high fat diet showed no significant change in adipose tissue weight compared to high fat diet alone.

Long Term Effect of Noni Juice Fasting Glucose Levels. Glucose levels in the blood after an overnight fast was measured at the start of the animal experiment, as well as at 4, 6, and 12 weeks. Figure 4.1b shows the compiled data. At day 0 of the experiment and at 4 weeks, there were no significant differences between all groups. After 6 and 12 weeks of noni treatment, there were no differences in the Con, Con + CJ, and Con + HJ. Circulating blood glucose levels were significantly higher in the untreated HFD group, whereas the HFD + CJ and HFD + HJ were not significantly greater than Con. The HFD + CJ and HFD + HJ groups displayed significantly reduced fasting blood glucose levels when compared to HFD alone (Fig 4.1b, $p < 0.05$).

Glucose Tolerance Test and Insulin Tolerance Test. Glucose tolerance tests were administered at 4 and 11 weeks into the study. At 4 weeks there were no significant differences between the untreated and noni treated mice on a control diet. The blood glucose levels in the HFD, HFD + CJ, and HFD + HJ were not statistically different at 30 and 60 minutes after injection when compared to Con. However, after 120 minutes the HFD and HFD + HJ groups had significantly elevated glucose levels compared to Con, whereas HFD + CJ was not. Interestingly, there were no statistically significant reductions in blood glucose in high fat diet fed mice treated with noni compared to HFD with out treatment (Figure 4.2a, $p < 0.05$). At 11 weeks, the glucose tolerance test was administered

again. Con + CJ and Con + HJ showed no significant difference when compared to Con. HFD and HFD + CJ exhibited significantly increased blood glucose levels at different time points, however HFD + HJ showed no significant differences compared to control. After injection, HFD had a 20, 27 and 43% increase in blood glucose levels at 30, 60 and 120 minutes, respectively. HFD + CJ showed no significant difference at 30 and 60 minutes, but had significant 36% increase in glucose levels at 120 minutes compared to control (Figure 4.2b, $p < 0.05$). At 30 minutes after injection, HFD + CJ was not significantly lower than HFD alone, whereas HFD + HJ was. At 60 and 120 minutes the blood glucose levels were slightly, but not significantly decreased compared to HFD alone.

Insulin tolerance tests were administered at 4 and 11 weeks following a 4 h fast. At 4 weeks Figure 4.3a shows that there were no observed changes in the animals' response to insulin in any groups when compared to control. After 11 weeks of treatment there were no significant differences between treated and untreated control groups. However, circulating glucose levels were higher in all high fat diet mice at 0h after the 4h fast. 30 minutes after insulin injection, the glucose levels in HFD and HFD + CJ were significantly higher than control. At 60 and 120 minutes only the untreated HFD group had significantly elevated glucose levels when compared to control (Figure 4.3b, $p < 0.05$).

Long Term Effects of Noni Juice on Plasma and Hepatic Triglyceride and Cholesterol. TG measured in the plasma was significantly reduced by 22% in Con + CJ mice, but no change was observed in Con + HJ mice. All groups consuming a high fat diet had significantly increased plasma TG compared to untreated control. HFD, HFD + CJ, and HFD + HJ displayed a 68%, 67%, and 100% increase in triglyceride, respectively (Figure 4.4a, $p < 0.05$). There were no statistically significant differences between HFD + CJ and HFD + HJ compared to HFD alone. There was however a 32% increase in HFD + HJ compared to HFD though it was not significant. Figure 4.4b shows that CH levels in all groups on a control diet did not have any significant differences when compared to the untreated control group. On the other hand, the HFD, HFD + CJ, and HFD + HJ were 291, 244 and 258% of control, respectively (Figure 4.4b, $p < 0.05$). CH levels in HFD + CJ and HFD + HJ were reduced by 47% and 33% (respectively) when evaluated against HFD alone, but it was not statistically significant.

Lipids were extracted from liver tissues and measured for TG and CH. When compared to the untreated control groups, there was up to 30% reduction in TG in Con + CJ and Con + HJ, but due to high variability between groups, these changes were not significant. However, HFD, HFD + CJ, and HFD + HJ had a 90%, 60%, and 61% increase in liver TG levels when compared to control (Figure 4.5a, $p < 0.05$). The high fat diet mice treated with noni up to 40% reduction in TG compared to HFD, but it was not statistically significant. There were also no

significant differences in total CH extracted from liver tissues. CH in treated control groups and high fat diet groups ranged from 97% to 107% of untreated control, showing that the variations between groups were not significant. (Figure 4.5b).

Western blot analysis of circulating apolipoproteins and PPAR γ . ApoA1 and ApoB100 were measured in the mouse plasma and PPAR γ was measured in adipose tissue. The levels of ApoA1 and ApoB100 were both slightly increased in the high fat diet fed mice when compared to control, however the results were not significant. There were no observed changes in PPAR γ between any groups. The data is not shown due to insufficient sample size. Only three batches from the seven were analyzed, and further investigation is warranted.

Long Term Effects of Noni Juice on Circulating Free Fatty Acids. The levels of circulating FFA in the plasma of the mice was not significantly affected by the diet or treatment with noni (Figure 4.7a).

Long Term Effects of Noni Juice on Adipokine Secretion. Adiponectin, resistin, and leptin was measured from mouse plasma. Figure 4.7b shows the adiponectin concentration in the plasma. The differences between the Con and Con +CJ or Con + HJ were statistically insignificant showing only a 6% increase

in both treated groups compared to Con. On the other hand, HFD, HFD +CJ, and HFD + HJ were all significantly reduced by up to 78% of control ($p<0.05$). There were no significant variations between HFD and treated high fat diet groups. Figure 4.8a shows the resistin concentrations in the plasma of the mice. There were slight increases (115% and 113%) in circulating resistin levels in Con + CJ and Con + HJ when measured against the untreated control group, although it was not significant. The mice fed a high fat diet displayed a significant 267, 271 and 262% increase in resistin when compared to Con ($p<0.05$) and there were only negligible differences between the untreated and treated high fat diet groups. Leptin was also measured in the mouse plasma. There were some trivial differences between the untreated and treated control groups. The Con + CJ had a 12% increase, whereas the Con + HJ group had a 24% decrease, however these changes were not significant due to high variation between groups. The mice fed a high fat diet all had dramatically significant increases in leptin levels, demonstrating a 1126, 967, and 1174% (HFD, HFD + CJ, and HFD + HJ, respectively) of untreated control. The changes observed between the treated and untreated high fat diet fed mice were not statistically significant when compared to HFD alone (Figure 4.7b, $p<0.05$).

TABLE 4.1

Long Term effects of noni juice consumption on body weight, food intake, and adipose tissue weight in C57BL/6J mice

| Group | Body Weight | | Feed Intake (kcal/day) | Adipose Weight (g) |
|----------|--------------------------|--------------------------|---------------------------|--------------------------|
| | Final (g) | Gained (g) | | |
| Control | 26.1 ± 2.07 | 3.04 ± 0.67 | 15.4 ± 0.98 | 0.98 ± 0.32 |
| Con + CJ | 24.1 ± 0.86 | 1.96 ± 0.52 | 14.7 ± 1.08 | 0.95 ± 0.23 |
| Con + HJ | 24.9 ± 1.47 | 2.37 ± 0.52 | 15.0 ± 0.85 | 0.94 ± 0.29 |
| HFD | 31.4 ± 2.36 ^b | 8.26 ± 1.96 ^b | 10.9 ± 0.62 ^a | 3.53 ± 1.34 ^b |
| HFD + CJ | 29.5 ± 0.97 ^b | 6.78 ± 0.92 ^b | 10.4 ± 0.49 ^a | 3.55 ± 0.54 ^b |
| HFD + HJ | 29.1 ± 2.90 ^b | 7.08 ± 1.16 ^b | 10.5 ± 0.51 ^a | 3.42 ± 1.01 ^b |

Data are represented as mean values ± SD (n=6), p≤0.05.

a = significant reduction compared to Con

b = significant increase compared to Con

c = significant decrease compared to HFD

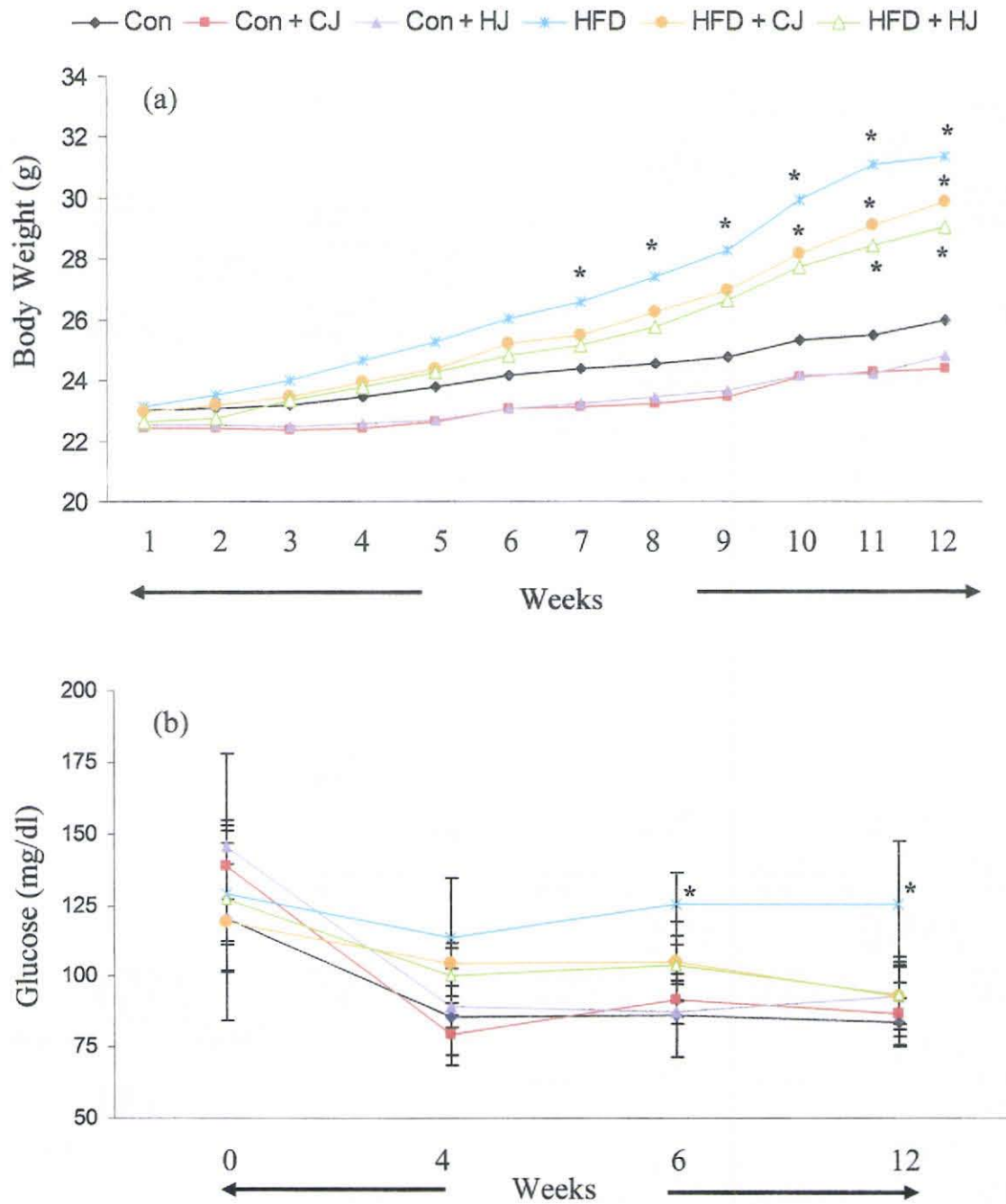


Figure 4.1: Effects of noni juice consumption on body weight (a) and fasting blood glucose levels (b) in C57BL/6J mice. Data are represented as mean values \pm SD (n=6), $p \leq 0.05$.

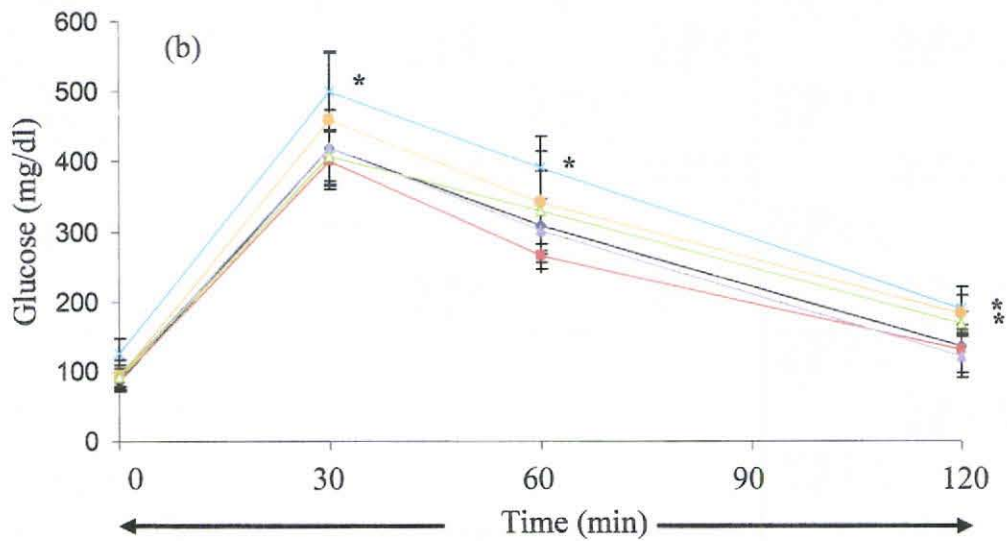
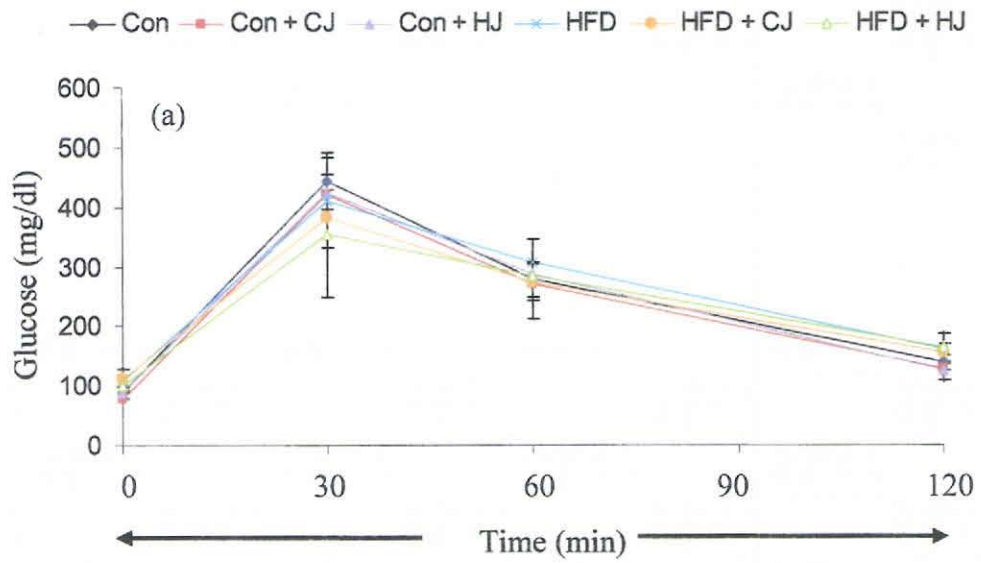


Figure 4.2: Effects of noni juice consumption on glucose tolerance at 4 weeks (a) and at 12 weeks (b) in C57BL/6J mice. Data are represented as mean values \pm SD (n=6), $p \leq 0.05$.

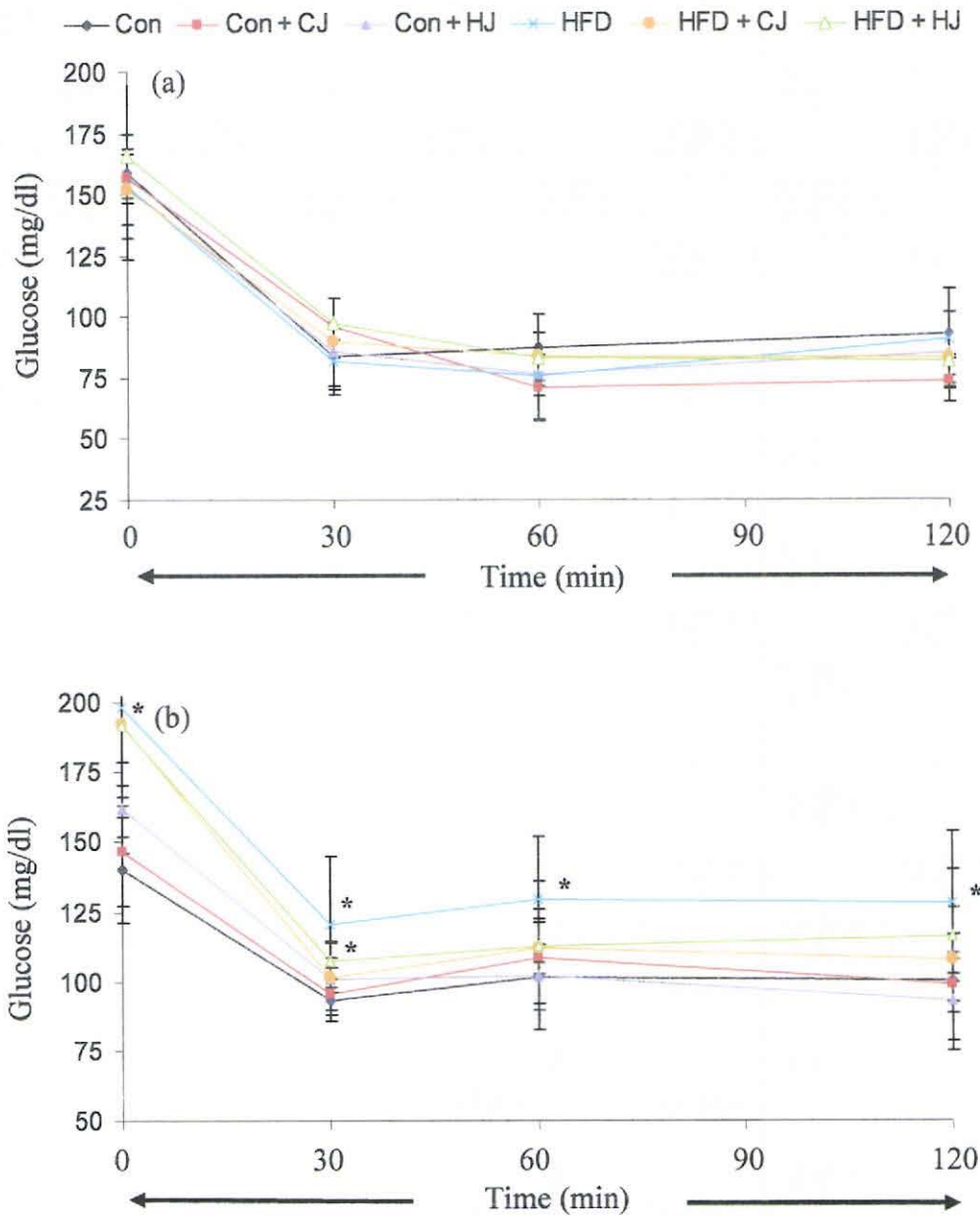


Figure 4.3: Effects of noni juice consumption on insulin tolerance at 4 weeks (a) and at 12 weeks (b) in C57BL/6J mice. Data are represented as mean values \pm SD (n=6), $p \leq 0.05$.

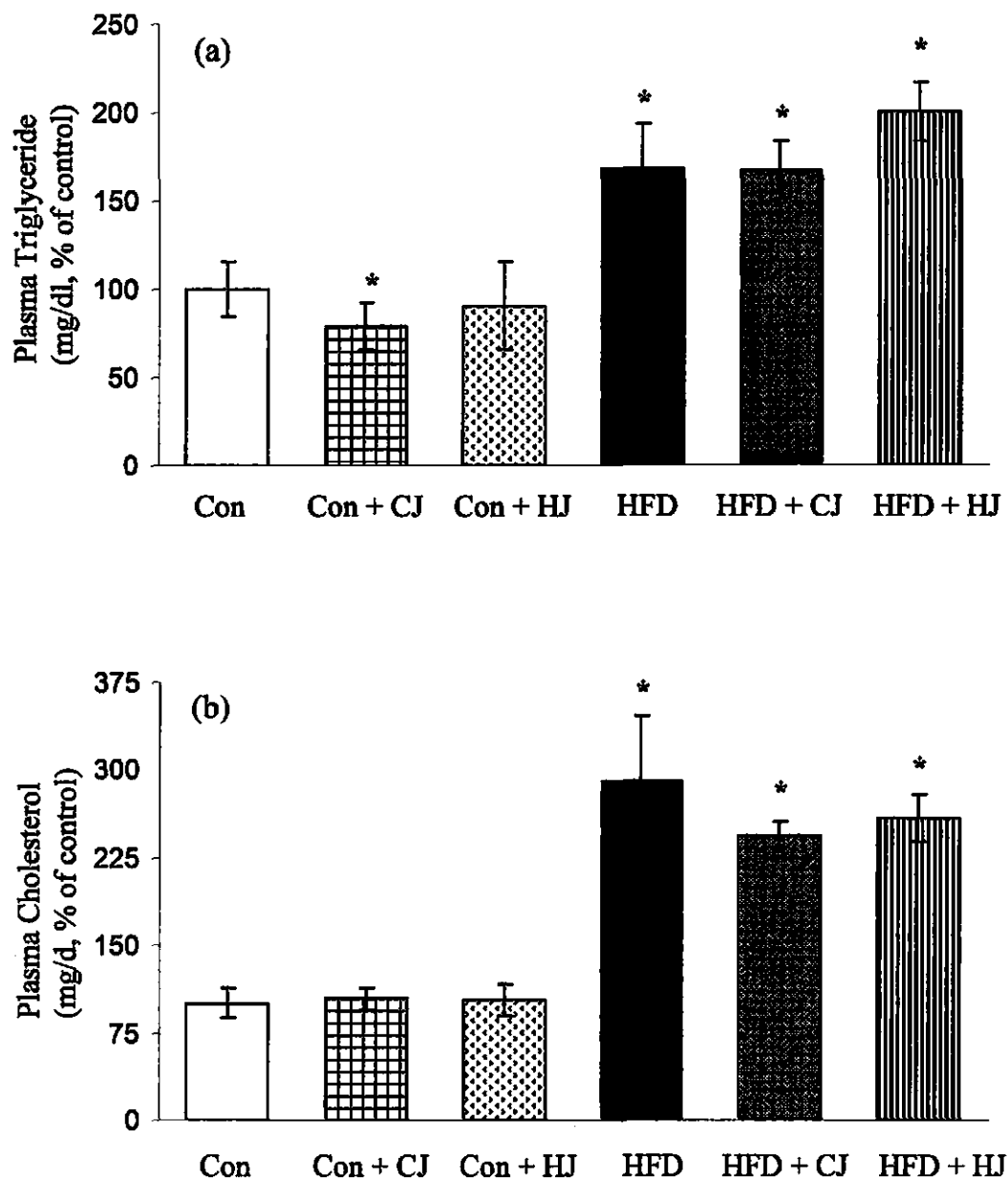


Figure 4.4: Effects of noni juice consumption on plasma triglyceride (a) and cholesterol levels (b) in C57BL/6J mice. Data are represented as a percentage of the control (set as 100%). Values are \pm SD (n=6), $p \leq 0.05$.

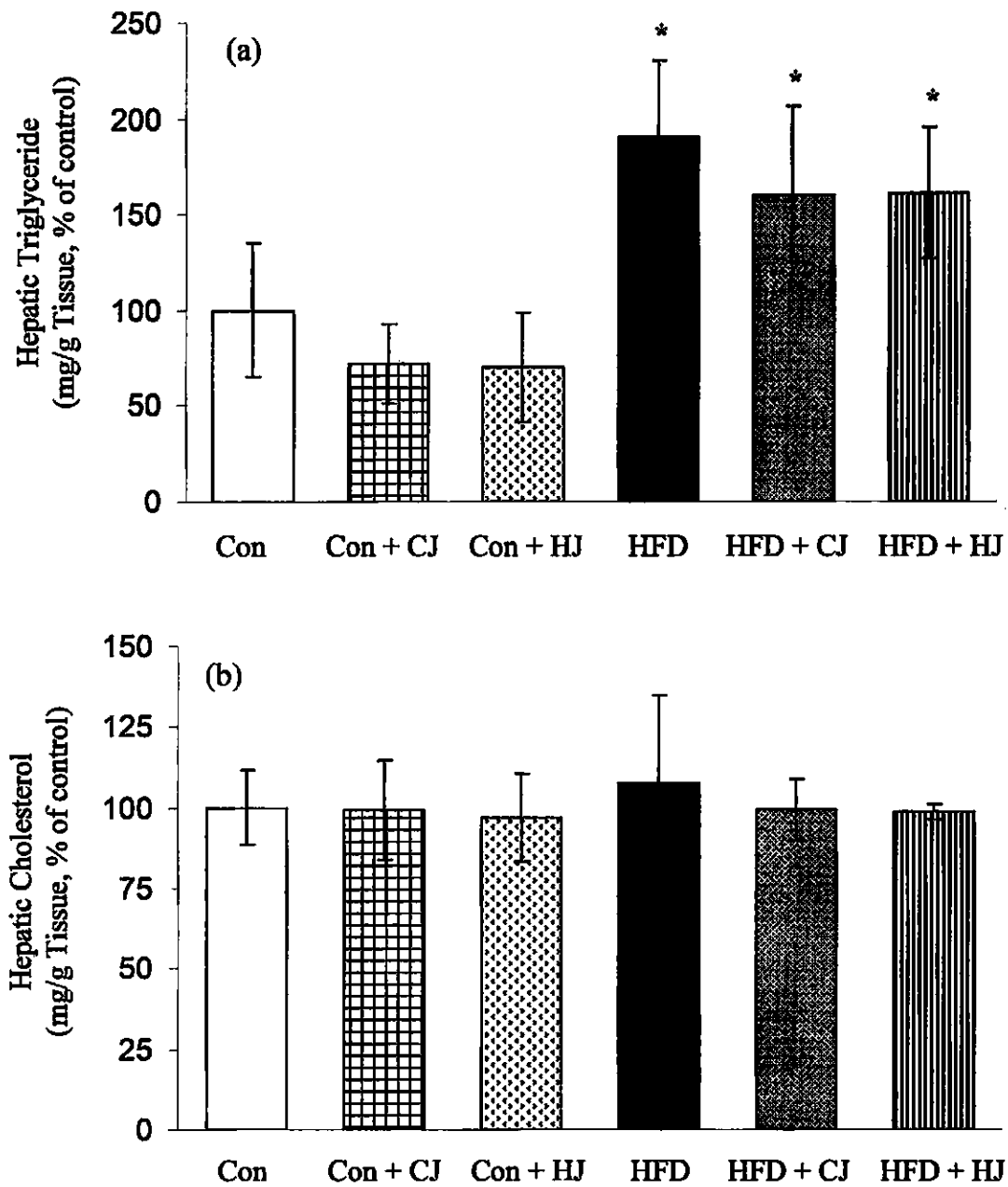


Figure 4.5: Effects of noni juice consumption on hepatic triglyceride (a) and cholesterol (b) in C57BL/6J mice. Data are represented as a percentage of the control (set as 100%). Values are \pm SD (n=5), $p < 0.05$.

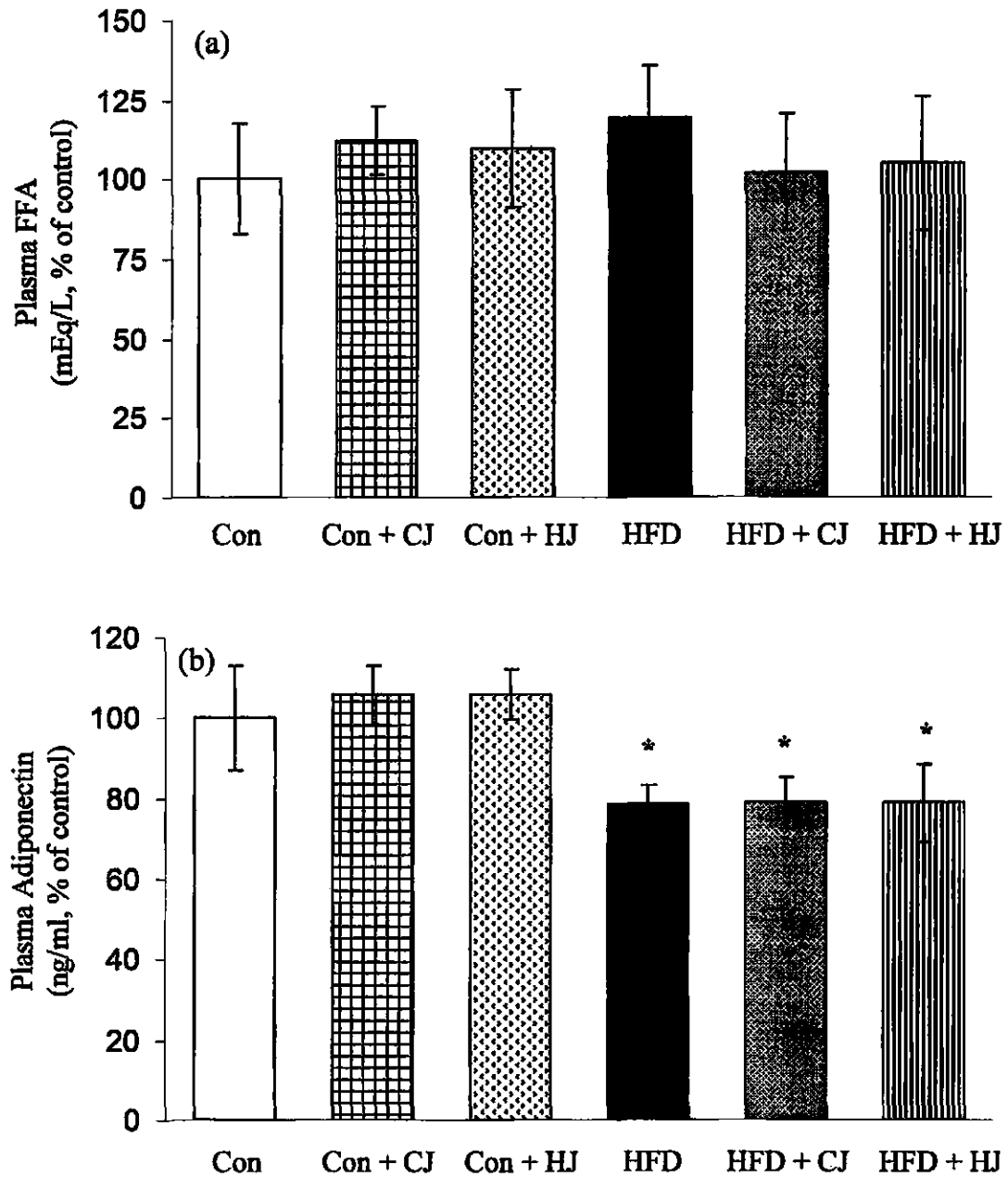


Figure 4.6: Effects of noni juice consumption on plasma free fatty acid (a) and plasma adiponectin (b) in C57BL/6J mice. Data are represented as a percentage of the control (set at 100%). Values are means \pm SD (n=5) and (n=4) $p < 0.05$ respectively.

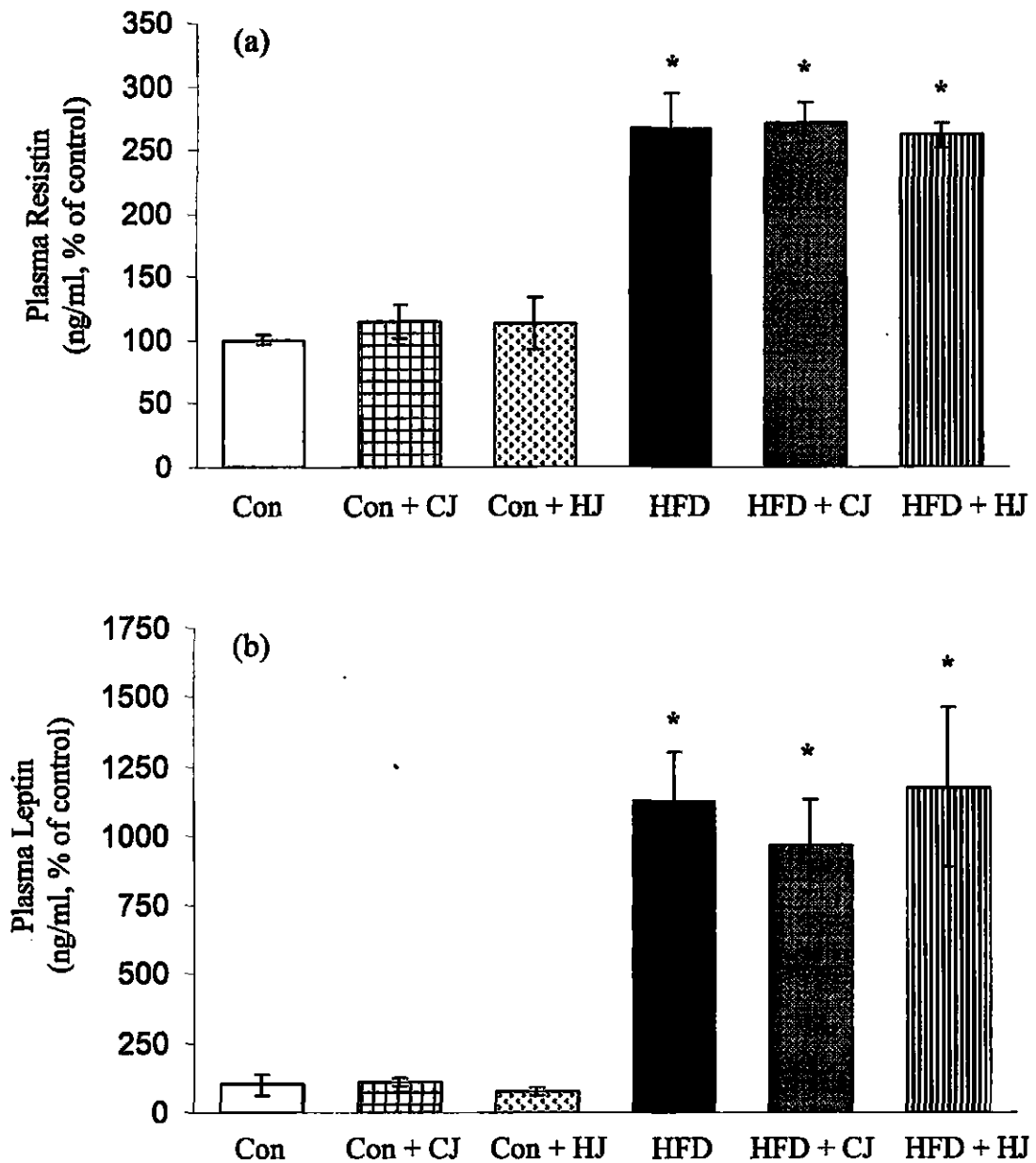


Figure 4.7: Effects of noni juice consumption on plasma resistin (a) and leptin (b) in C57BL/6J mice. Data are represented as a percentage of the control (set at 100%). Values are means \pm SD (n=4), $p < 0.05$.

DISCUSSION

In this study, we examined the effects of a commercial noni juice and homemade noni juice in C57BL/6J mice over a 12 week period with focus on lipid and glucose metabolism. Our results showed that the body weight of the control mice were not significantly affected by noni treatment. However, the high fat diet mice that were treated with noni juice took several weeks longer to weigh significantly more than control. This could indicate that the noni juice had some effect on slowing the rate of weight increase. The mice fed a high fat diet had significantly more fat indicating that the mice did indeed become obese. The body weight and fat weight was not affected by the food intake.

The high fat diet mice treated with either commercial or homemade juice both displayed improved glucose tolerance and improved insulin sensitivity by the end of the 12 week study. The high fat diet fed mice that were treated with noni juice also exhibited decreased levels of glucose in the blood after an over night fast. These findings indicate that both types of noni juice have hypoglycemic properties and have the ability to positively regulate glucose metabolism *in vivo*. Similarly, Nayak et al. (2007) have previously shown that fasting glucose levels in the diabetic induced mice had a 29% reduction in fasting glucose levels compared to control diabetic mice when treated with noni juice.

The noni juice did not have any effect on the adipocytokines secreted from the adipose tissue. Adipocytokines appear to be at the interface of

cardiometabolic disease, and PPARs have been demonstrated to have important effects on their expression. The adipocytokines released from adipose tissue, participate in the development of insulin resistance, but may also modulate endothelial dysfunction, and promote atherosclerosis (Lau et al., 2005). The adiponectin, leptin, and resistin levels were not significantly different in the noni juice treated groups. There were however, differences in the high fat diet groups compared to control. The adiponectin levels in the high fat diet groups were significantly reduced. It has been shown that adiponectin levels are inversely proportional to body weight, meaning that there is a decrease in adiponectin when there is an increase in body weight. A decrease in adiponectin is also associated with impaired glucose uptake (Yamauchi et al., 2001). Although glucose uptake was not measured, it is evident that adiponectin was not the primary mechanism in improving glucose tolerance in noni juice treated mice, because the treated mice showed no change compared to control, and the high fat diet treated mice showed no increase in adiponectin levels compared to high fat diet alone.

Under normal physiological conditions, the production of leptin by adipose is directly correlated to the mass of the tissue (Campfield *et al.*, 1996). In our study we did find that the leptin levels in the high fat diet mice were significantly higher than the control groups. Studies conducted on circulating leptin has been shown to affect insulin sensitivity and glucose metabolism, however in our study there were no significant changes between the high fat diet treated mice and the

untreated mice, indicating that leptin may not affect the reduced blood glucose levels in the treated mice (Cohen *et al.*, 1996, Liu *et al.*, 1997).

Resistin has also been linked to diabetes and obesity. This relationship is supported by studies showing that there is an increase in blood glucose when resistin is administered to rodents (Banerjee *et al.*, 2004). Mice lacking resistin also exhibit low blood glucose, and diminishes the increase in post-fast blood glucose typically associated with obesity. This suggests that resistin has a role in mediating hyperglycemia associated with obesity. In our study, resistin was significantly increased in the high fat diet mice when compared to control. However, there were no differences between high fat diet alone or with noni juice treatment. Therefore, it is indicative that the noni juice treatment does not significantly affect the secretion of adipocytokines and the observed regulation of glucose metabolism must be through different mechanisms.

Dyslipidemia is characterized by increased levels of very low-density lipoprotein (VLDL), small dense LDL particles, triglycerides, cholesterol and total cholesterol, and a decrease in high density lipoprotein (HDL) cholesterol levels. Our study indicates that commercial noni juice may beneficially affect the lipids metabolism. In this study, we found that the plasma triglyceride levels were significantly reduced by 22% in the mice treated with commercial noni juice but not with homemade juice. There were no significant differences between the high fat diet groups though there were significant increases when compared to control.

There were also dramatic decreases in hepatic triglyceride in mice treated with noni juice, but it was not significant due to the high variability between samples. There were no changes observed in the plasma cholesterol levels in the control mice when treated with commercial or homemade noni juice. However, there were some decreases in cholesterol when comparing the high fat diet groups. Treatment with noni juice did reduce plasma cholesterol, however it was not significant. There were no observed changes in hepatic cholesterol between all groups. To diagnose dyslipidemia, individual lipoproteins are often measured along with triglyceride and cholesterol levels. In our study we found that the high fat diet groups had increased levels of apoA1 and apoB, due to the insufficient sample size it is not clear whether noni juice regulated apolipoprotein secretion. The levels of FFA were also unchanged in the treated and untreated groups on a control and high fat diet. This could be due to the high standard deviation between the samples.

In summary, both commercial and homemade noni juice did have hypoglycemic properties, and may have some hypolipidemic properties. However, it did not have any beneficial effects on regulating the adipocytokines or FFA. Further studies to investigate the use of noni juice as an anti-diabetic agent is warranted.

GENERAL DISCUSSION

In the animal model, short term treatment with noni juice (5 weeks) was able to reduce the body weight of the treated mice. However, whether the weight loss was a bodily reaction to the noni juice or the reduced caloric consumption is still unclear. There were also some slight reductions in fasting glucose levels in the short term study, but these were not significant. On the other hand, in the long term study (12 weeks) the blood glucose levels after an overnight fast were significantly reduced in the high fat diet treated mice when compared to high fat diet alone, and were not significantly different from the untreated control, showing that the noni juice treatment may have some fasting glucose lowering properties. In both the 5 week and 12 weeks study, the glucose tolerance test and insulin tolerance test displayed some modulation of glucose by noni juice. There were significant improvements in the glucose tolerance in noni juice treated mice after glucose was injected intraperitoneally and the same holds true for the insulin tolerance test conducted on the mice. In the 5 week study, TG, CH, adiponectin, and FFA were also moderately affected, however none of these changes were significant. The same holds true for the 12 weeks study. There were some modulatory effects of noni juice on lipid metabolism, such as the reduction of plasma and hepatic triglyceride levels, however these changes were not significant due to high standard deviations between samples. The effects of noni juice on

FFA and adipocytokines in both the 5 week and 12 weeks study did not show significant improvements. Therefore, it is suggested that noni juice may not alter the FFAs and adipocytokines, and the glucose improvement may be affected by molecules that were not measured in this study.

Based on the 5 weeks study, we decided to investigate the effects of noni juice *in vitro* to see if the effects expressed in the animal tissue would correlate to the individual cell type. Since there were slight modifications in the triglyceride and cholesterol in the liver and plasma, HepG2 cells were used. In these studies we found there to be significant reductions in cellular triglyceride and cellular cholesterol mass. This however was only evident in cells that were not in the presence of excess of lipids.

In the mouse model, weight gain in the treated animals were significantly less than the control animals. Therefore, we decided to investigate the effects of noni as an anti-obesity agent and used 3T3-L1 adipocytes. Interestingly, we found that the cells treated with noni juice after adipogenesis significantly reduced cellular triglyceride mass. However, when cells were treated during adipogenesis, there were significant increases in cellular triglyceride. These results contradict the results observed in the 5 weeks animal study. One reason for this is that in culture, the noni juice is only acting on one type of tissue in this case adipose. However, in the animal, it is acting on the whole system and the noni juice could affect other tissues and may be metabolized differently when ingested.

REFERENCES

- Ahmad, V. U., and Bano, S. (1980) Isolation of beta-sitosterol and ursolic acid from *Morinda citrifolia* Linn. *J Chem Soc Pak*, **2**, 71.
- Arad, Y., Ramakrishnan, R. and Ginsberg, H. N. (1990) Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J Lipid Res*, **31**, 567-582.
- Atkinson, N. (1956) Antibacterial substances from flowering plants. 3. Antibacterial activity of dried Australian plants by a rapid direct plate test. *Aust J Exp Biol Med Sci*, **34**, 17-26.
- Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., Rich, A. S., Rhoades, B., Qi, Y., *et al.* (2004) Regulation of fasted blood glucose by resistin. *Science*, **303**, 1195-1198.
- Barnes, P. M., Powell-Griner, E., McFann, K. and Nahin, R. L. (2004) Complementary and alternative medicine use among adults: United States, 2002. *Adv Data*, 1-19.
- Bays, H., Mandarino, L. and DeFronzo, R. A. (2004) Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab*, **89**, 463-478.
- Bjorbaek, C. and Kahn, B. B. (2004) Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res*, **59**, 305-331.
- Boren, J., Rustaeus, S. and Olofsson, S. O. (1994) Studies on the assembly of apolipoprotein B-100- and B-48-containing very low density lipoproteins in McA-RH7777 cells. *J Biol Chem*, **269**, 25879-25888
- Bowie, J. H., Cooke, R. G. (1962) Colouring matters of Australian plants. IX. Anthraquinones from *Morinda* species, **15**, 332.
- Campfield, L. A., Smith, F. J. and Burn, P. (1996) The OB protein (leptin) pathway—a link between adipose tissue mass and central neural networks. *Horm Metab Res*, **28**, 619-632.
- Castelli, W. P., Garrison, R. J., Wilson, P. W., Abbott, R. D., Kalousdian, S. and Kannel, W. B. (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA*, **256**, 2835-2838.

- Chandran, M., Phillips, S. A., Ciaraldi, T. and Henry, R. R. (2003) Adiponectin: more than just another fat cell hormone? *Diabetes Care*, **26**, 2442-2450.
- Cohen, B., Novick, D. and Rubinstein, M. (1996) Modulation of insulin activities by leptin. *Science*, **274**, 1185-1188.
- Combs, T. P., Berg, A. H., Obici, S., Scherer, P. E. and Rossetti, L. (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest*, **108**, 1875-1881.
- DeFronzo, R. A., Gunnarsson, R., Bjorkman, O., Olsson, M. and Wahren, J. (1985) Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest*, **76**, 149-155.
- Dixon, A. R., McMillen, H. and Etkin, N. L. (1999) Ferment this: the transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). *Ecological Botany*, **53**, 51-68.
- Dixon, J. L. and Ginsberg, H. N. (1993) Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: information obtained from cultured liver cells. *J Lipid Res*, **34**, 167-179.
- Dunstan, D. W., Zimmet, P. Z., Welborn, T. A., De Courten, M. P., Cameron, A. J., Sicree, R. A., *et al.* (2002) The rising prevalence of diabetes and impaired glucose tolerance: the Australian Diabetes, Obesity and Lifestyle Study. *Diabetes Care*, **25**, 829-834.
- Eckel, R. H., Grundy, S. M. and Zimmet, P. Z. (2005) The metabolic syndrome. *Lancet*, **365**, 1415-1428.
- Farine, J. P., Legal, L., Moreteau, B. and Le Quere, J. L. (1996) Volatile Compounds of Ripe Fruits of *Morinda Citrifolia* and Their Effects on *Drosophila*. *Phytochemistry*, **41**, 433-438.
- Fielding, C. J., Havel, R. J., Todd, K. M., Yeo, K. E., Schloetter, M. C., Weinberg, V., *et al.* (1995) Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. *J Clin Invest*, **95**, 611-618.
- Goldstein, J. L. and Brown, M. S. (1990) Regulation of the mevalonate pathway. *Nature*, **343**, 425-430.
- Guri, A. J., Hontecillas, R. and Bassaganya-Riera, J. (2006) Peroxisome proliferator-activated receptors: bridging metabolic syndrome with molecular nutrition. *Clin Nutr*, **25**, 871-885.

- Heinicke, R. M. (1985) The Pharmacologically Active Ingredient of Noni. *Pacific Tropical Botanical Garden Bulletin*, **15**, 10-14.
- <http://nonipower.blogspot.com/2006/04/noni-dosage.html>.
- <http://www.noni-is-good-for-you.com/>.
- Hulthe, J., Hulten, L. M. and Fagerberg, B. (2003) Low adipocyte-derived plasma protein adiponectin concentrations are associated with the metabolic syndrome and small dense low-density lipoprotein particles: atherosclerosis and insulin resistance study. *Metabolism*, **52**, 1612-1614.
- Isomaa, B., Almgren, P., Tuomi, T., Forsen, B., Lahti, K., Nissen, M., *et al.* (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*, **24**, 683-689.
- Jahromi, M. A. and Ray, A. B. (1993) Antihyperlipidemic effect of flavonoids from *Pterocarpus marsupium*. *J Nat Prod*, **56**, 989-994.
- Julius, U. (2003) Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. *Exp Clin Endocrinol Diabetes*, **111**, 246-250.
- Kamiya, K., Tanaka, Y., Endang, H., Umar, M. and Satake, T. (2004) Chemical constituents of *Morinda citrifolia* fruits inhibit copper-induced low-density lipoprotein oxidation. *J Agric Food Chem*, **52**, 5843-5848.
- Kazumi, T., Kawaguchi, A., Hirano, T. and Yoshino, G. (2004) Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. *Metabolism*, **53**, 589-593.
- Kershaw, E. E. and Flier, J. S. (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*, **89**, 2548-2556.
- Kinlaw, W. B. and Marsh, B. (2004) Adiponectin and HIV-lipodystrophy: taking HAART. *Endocrinology*, **145**, 484-486.
- Krauss, B. H. (1993) *Plants in Hawaiian Culture*. (ed. ^eds.). Hawaii: University of Hawaii Press, pp. 16.
- Langin, D. (2006) Control of fatty acid and glycerol release in adipose tissue lipolysis. *C R Biol*, **329**, 598-607; discussion 653-595.
- Lau, D. C., Dhillon, B., Yan, H., Szmítko, P. E. and Verma, S. (2005) Adipocytokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol*, **288**, H2031-2041.

- Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M. and Kliewer, S. A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem*, **270**, 12953-12956.
- Linde, K., ter Riet, G., Hondras, M., Vickers, A., Saller, R. and Melchart, D. (2001) Systematic reviews of complementary therapies - an annotated bibliography. Part 2: herbal medicine. *BMC Complement Altern Med*, **1**, 5.
- Liu, Y. L., Emilsson, V. and Cawthorne, M. A. (1997) Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (ob/ob) mice. *FEBS Lett*, **411**, 351-355.
- Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., *et al.* (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med*, **8**, 731-737.
- Matsubara, M., Maruoka, S. and Katayose, S. (2002) Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab*, **87**, 2764-2769.
- Maxfield, F. R. and Tabas, I. (2005) Role of cholesterol and lipid organization in disease. *Nature*, **438**, 612-621.
- McClatchey, W. (2002) From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integr Cancer Ther*, **1**, 110-120; discussion 120.
- McCoy, M. L. G., Thomas, E. A. and Simon, O. R. (2002) Preliminary Investigation of the Anti-inflammatory Properties of an Aqueous Extract from *Morinda Citrifolia* (Noni). *Pharmacological Society*, **45**, 76-78.
- Meier, U. and Gressner, A. M. (2004) Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem*, **50**, 1511-1525.
- Mokdad, A. H., Serdula, M. K., Dietz, W. H., Bowman, B. A., Marks, J. S. and Koplan, J. P. (1999) The spread of the obesity epidemic in the United States, 1991-1998. *JAMA*, **282**, 1519-1522.
- Mokdad, A. H., Serdula, M. K., Dietz, W. H., Bowman, B. A., Marks, J. S. and Koplan, J. P. (2000) The continuing epidemic of obesity in the United States. *JAMA*, **284**, 1650-1651.
- Moniz, H. (1992). US patent 5288491

- Morton, J. F. (1992) The ocean-going Noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorful" relatives. *Ecological Botany*, **46**, 241-256.
- Nayak, B. S., Isitor, G. N., Maxwell, A., Bhogadi, V. and Ramdath, D. D. (2007) Wound-healing activity of *Morinda citrifolia* fruit juice on diabetes-induced rats. *J Wound Care*, **16**, 83-86.
- Oral, E. A., Simha, V., Ruiz, E., Andewelt, A., Premkumar, A., Snell, P., *et al.* (2002) Leptin-replacement therapy for lipodystrophy. *N Engl J Med*, **346**, 570-578.
- Rana, J. S., Nieuwdorp, M., Jukema, J. W. and Kastelein, J. J. (2007) Cardiovascular metabolic syndrome - an interplay of, obesity, inflammation, diabetes and coronary heart disease. *Diabetes Obes Metab*, **9**, 218-232.
- Roden, M., Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W., *et al.* (1996) Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest*, **97**, 2859-2865.
- Roden, M., Stingl, H., Chandramouli, V., Schumann, W. C., Hofer, A., Landau, B. R., *et al.* (2000) Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. *Diabetes*, **49**, 701-707.
- Rosen, E. D., Sarraf, P., Troy, A. E., Bradwin, G., Moore, K., Milstone, D. S., *et al.* (1999) PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell*, **4**, 611-617.
- Ryo, M., Nakamura, T., Kihara, S., Kumada, M., Shibazaki, S., Takahashi, M., *et al.* (2004) Adiponectin as a biomarker of the metabolic syndrome. *Circ J*, **68**, 975-981.
- Siperstein, M. D., Jayko, M. E., Chaikoff, I. L. and Dauben, W. G. (1952) Nature of the metabolic products of C14-cholesterol excreted in bile and feces. *Proc Soc Exp Biol Med*, **81**, 720-724.
- Sparks, J. D. and Sparks, C. E. (1985) Apolipoprotein B and lipoprotein metabolism. *Adv Lipid Res*, **21**, 1-46.
- Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., *et al.* (2001) The hormone resistin links obesity to diabetes. *Nature*, **409**, 307-312.
- Su, C., Wang, M., Nowicki, D., Jensen, J. and Anderson, G. (2001) Selective Cox-2 Inhibition of *Morinda citrifolia* (Noni) in vitro. In: *Inflammation and Related Disease. The 7th Annual Conference.* (ed. ^eds.). Nashville, Tennessee.

- Tamori, Y., Masugi, J., Nishino, N. and Kasuga, M. (2002) Role of peroxisome proliferator-activated receptor-gamma in maintenance of the characteristics of mature 3T3-L1 adipocytes. *Diabetes*, **51**, 2045-2055.
- Tiwari, R. D., Singh, J. (1976) Structural study of the anthraquinone glycoside from the flowers of *Morinda citrifolia*. *J Indian Chem Soc*, **54**, 429-430
- Trujillo, M. E. and Scherer, P. E. (2005) Adiponectin—journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med*, **257**, 167-175.
- Vega, G. L., Denke, M. A. and Grundy, S. M. (1991) Metabolic basis of primary hypercholesterolemia. *Circulation*, **84**, 118-128.
- Vickers, A. and Zollman, C. (1999) ABC of complementary medicine: herbal medicine. *BMJ*, **319**, 1050-1053.
- Wang, C. C., Goalstone, M. L. and Draznin, B. (2004) Molecular mechanisms of insulin resistance that impact cardiovascular biology. *Diabetes*, **53**, 2735-2740.
- Wang, M. Y. and Su, C. (2001) Cancer preventive effect of *Morinda citrifolia* (Noni). *Ann N Y Acad Sci*, **952**, 161-168.
- Webber, J. (2003) Energy balance in obesity. *Proc Nutr Soc*, **62**, 539-543.
- Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., *et al.* (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nat Med*, **7**, 941-946.
- Yki-Jarvinen, H. (2004) Thiazolidinediones. *N Engl J Med*, **351**, 1106-1118.
- Zanlungo, S. and Nervi, F. (2003) Discovery of the hepatic canalicular and intestinal cholesterol transporters. New targets for treatment of hypercholesterolemia. *Eur Rev Med Pharmacol Sci*, **7**, 33-39.
- Zhang, Y. L., Hernandez-Ono, A., Ko, C., Yasunaga, K., Huang, L. S. and Ginsberg, H. N. (2004) Regulation of hepatic apolipoprotein B-lipoprotein assembly and secretion by the availability of fatty acids. I. Differential response to the delivery of fatty acids via albumin or remnant-like emulsion particles. *J Biol Chem*, **279**, 19362-19374.