**EFFECTS OF A NORTHERN CONTAMINANT MIXTURE, DIET, AND BODY
WEIGHT ON GLUCOSE AND CHOLESTEROL METABOLISM AND LIPOPROTEIN
SIGNALING IN JCR RAT LIVER.**

By

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Abstract:

Northern Canadian populations (specifically the Inuit) display higher prevalence of diabetes and cardiovascular diseases. It is believed that diet and lifestyle contribute to the development of these disease; however, recent epidemiological studies suggest that persistent organic pollutants and heavy metals may also contribute to the development of these diseases. Northern populations are exposed to these chemicals that bioaccumulate in the environment through food consumption. In this study, we outline the effects of these chemicals and the consumption of high fat/sugar food on glucose and cholesterol metabolism and lipoprotein signaling in JCR rat liver. Using gene profiler arrays, we have identified four genes that are affected mainly by these contaminants and/or diet. Our results indicate that these mixtures alter the expression of genes in gluconeogenesis pathway (fructose 1,6-bisphosphatase and pyruvate carboxylase), a protein involved in cholesterol transport (apolipoprotein A1), and a transcription factor that is responsible for the expression of proteins in lipogenesis pathway (sterol regulatory element binding transcription factor 1). These proteins could also play an important role in insulin resistance and atherosclerosis; therefore, it is important to follow up our experiment in order to understand the increased risk of diabetes and cardiovascular diseases found in Northern Canadian populations.

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List of Abbreviations:

APOA1: Apolipoprotein A1

ATP: Adenosine Triphosphate

CHD: Coronary Heart Disease

DDE: Dichlorodiphenyldichloroethylene

DDT: Dichlorodiphenyltrichloroethane

ELISA: Enzyme-linked Immunosorbent Assay

eNOS: Endothelial Nitric Oxide Synthase

FBP2: Fructose 1,6-Bisphosphatase

HDL: High Density Lipoprotein

JCR: J.C. Russel of University of Alberta

LDL Low Density Lipoproteins

LXR: Liver X Activated Receptors

MAPK: Mitogen Activated Protein Kinase

NAFLD: Non-alcoholic Fatty Liver Disease

NCM: Northern Contaminant Mixture

P450_{scc}: Cytochrome p450 Side Chain Cleavage

PBDE: Polybrominated diphenyl ethers

PC: Pyruvate carboxylase

PCB: Polychlorinated biphenyls

PFOS: Perfluorooctane

POPS: Persistent Organic Pollutants

PPAR γ : Peroxisome Proliferator-activated Receptor γ

PPRE: Peroxisome Proliferator Response Element

RT-RT-PCR: Reverse Transcription Real-Time Polymerase Chain Reaction

SD: Standard Deviation

SEM: Standard Error of Means

SRE: Sterol Regulatory Element

SREBF1: Sterol Regulatory Element Transcription Factor

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Chapter 1: Introduction

1.1 General Overview

Northern Canadian populations are exposed to environmental contaminants such as heavy metals, methylmercury, cadmium, polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs). These contaminants are carried by oceanic and wind currents from the southern to the northern latitude and integrate with the bio environment in the Arctic (Dewailly et al. 2006). Recent experimental studies have shown that these chemicals may play a role in the development and progression of cardiovascular and metabolic disorders; however, it still remains unclear of the exact mechanism. The prevalence of diabetes is higher in First Nations individuals when compared to the general Canadian population (3.6 times higher for men and 5.3 times higher for women) (Ralph-Campbell et al, 2009). The prevalence of obesity in Inuit population is 3% more than the general Canadian population (24.27% and 21.05%, respectively) (Bruce et al., 2014). There is also an observed 14% increase in obesity rates in Nunavut Inuit between 1992 and 2004 (Anctil, 2004). Moreover, circulatory diseases are emerging as a leading cause of death in populations of the Canadian North. This can partially be attributed to the changes in diets and lifestyles. Traditional diets of Inuit included any food available in their areas such wild animals, fish, marine mammals, and wild berries among others (Sheeshy et al., 2013). In the last couple of decades however, western society lifestyle have influenced Inuit lifestyle that resulted in the consumption of more processed/ store bought foods that were not available (Sheeshy et al., 2013). This change in lifestyle and diet brought health concerns for Inuits as they are consuming more sugar and fat rich foods (Sharma, 2010; and Kuhnlein et al., 2007). However, recent evidence suggests that chronic exposure to these Northern contaminants may play a role in cardiovascular diseases (Florian et al. 2013).

Our ongoing study looks at the effects of a Northern contaminant mixture (NCM; 23 chemicals) outlined in (Table. 1), diet, and body weight on cholesterol and glucose metabolism in J.C. Russell (JCR, University of Alberta) rat liver. Most of these chemicals are present in higher concentrations in Inuit's blood than in the general Canadian population (Table 2). Diet is believed to be a significant source of exposure to these chemicals due to the accumulation of the lipophilic chemicals in the fat tissues of their consumed animals (Table 4). The current hypothesis is that Northern contaminant mixture may contribute to the development and progression of metabolic and cardiovascular diseases. One objective of this study is to assess the effects of these chemicals on cholesterol and glucose metabolism in JCR rat liver, and monitor protein/lipoprotein synthesis and transport (Figure 1). We also speculate that these effects of contaminant mixtures may be modulated by dietary and lifestyle factors such as high fat/sugar consumption.

The impact upon the health of Inuit of these contaminants remains unclear. Our study tries to understand the changes that happen at the gene and protein level as a response to the collective administration of 23 Northern contaminants to JCR rats. Using Reverse Transcription-Real Time Polymerase Chain Reaction (RT-PCR), we examined changes in the expression of 82 genes that are involved in cholesterol and lipoprotein metabolism, and another 82 different genes that have a role in glucose metabolism as a result of NCM administration. Further confirmation at the protein level is necessary to try to understand the effects of NCM. However, the result of this study cannot be attributed to a single chemical or molecule because the effects of one chemical in the NCM may be altered by the presence of the other (Florian et al. 2009). The overall objective of the study is to determine if NCM plays a role in the development of diseases related glucose and cholesterol metabolism and lipoprotein signaling.

1.2 NCM in Literature:

1.2.1 Heavy metals

Heavy metals are probably the oldest toxins known as they are natural constituents of Earth's crust. Human exposure to heavy metals comes from many sources such as contaminated food, commercial products, and natural sources. The toxicity of heavy metals depends on the amount of exposure. Heavy metals affect different organ systems, most notably the central nervous, cardiovascular, and gastrointestinal systems (Sharma et al. 2014).

1.2.1.1 Mercury

Mercury is present in different forms which include organic mercury compounds, inorganic mercury, and mercury vapour. Methylmercury (MeHg) is the bio transformed form of mercury; in which sulfate reducing bacteria methylate elemental mercury in aquatic environments. As a result, MeHg enters the food chain and accumulate in higher order of predatory animals, mainly marine fish and mammals (Ceccatelli et al. 2010). The exact mechanism of mercury toxicity is not fully understood but it is widely accepted that it binds to thiol groups such as glutathione and metallothioneins (Fitzgerald and Clarkson, 1991). Epidemiological studies conducted in the Amazon and Finland suggests that the risk of cardiovascular disease increases through MeHg exposure (Fillion et al. 2006; Stern, 2005), but the mechanism of which remains unclear. A birth cohort study in Faroe Island found an association between increased systolic and diastolic blood pressure and prenatal exposure to increasing methylmercury concentrations (Sorensen et al. 1999). It also found decreased heart rate variability in 7 year old children, in which children with lower

birth weight saw stronger mercury effects (Sorensen et al. 1999). Another study conducted in Nunavik found that after adjusting for confounding factors, diastolic and systolic blood pressure was positively and significantly correlated with mercury levels in blood (Velara et al. 2008). Another study showed that rats developed systemic hypertension when exposed chronically to methylmercury (Wakita, 1987). Furthermore, endothelial dysfunction and an increase in oxidative stress are observed as a result of chronic exposure to low concentrations of methylmercury (Wiggers et al. 2008).

1.2.1.2 Cadmium

Earth's crust has naturally occurring cadmium, both as elemental and mineral form. Smoking is a major source of cadmium exposure because tobacco has a significant amount of it. Since cadmium is an elemental metal, it cannot be degraded. Its main use is in rechargeable batteries, plastics, paints, and in the smelting industry. Cadmium contaminates agricultural soil and is taken up by edible plants, making it another source of human exposure.

Cadmium's toxicity has been studied for a long time, and its targets include lungs, kidney, endothelium, and liver. Studies have shown cadmium's association with many cardiovascular diseases such as hypertension (Varoni et al. 2003) and cardiomyopathy (Kopp et al. 1978). Endothelial nitric oxide synthase (eNOS) is one target protein for cadmium in that it inhibits eNOS phosphorylation resulting in decreased NO production in endothelial hybrid cell line EAhy926 (Majumder et al. 2008). It has also been shown to induce apoptosis in mouse brain endothelial cells by stimulating p38 mitogen-activated protein kinase (MAPK) (Jung et al. 2008).

1.2.1.3 Lead

One of many toxic elements, lead is naturally found in volcanic activities and in ores. It is used in building materials, paints, batteries, gasoline, bullets, and radiation shields. Human exposure to lead is mainly through emissions in air, workplace exposure, food and drink cans, and gasoline additives. Lead is able to enter the food chain as airborne lead can set on land and water masses, and contact with waste deposits (Papanikolaou et al. 2005). Lead is absorbed through skin, ingestion, and inhalation. It affects most of the human body systems such as the kidneys, the central nervous system, liver, and cardiovascular system. The human body does not metabolize the inorganic form but is able to metabolize the organic form through oxidation by cytochrome P-450 (Papanikolaou et al. 2005).

One of lead's toxicity mechanisms relies on inhibiting three enzymes responsible for synthesizing heme (Piomelli, 2002). Lead levels in blood and bones show stronger association with increased cardiovascular mortality (Navas-acien et al. 2004). Another study has shown that treating vascular smooth muscle cells and human coronary endothelial cells with lead acetate has significantly increased the production of hydrogen peroxide and superoxide suggesting that lead-oxidative stress involvement which could result in endothelial dysfunction (Ni et al. 2004). Other studies have shown that exposure to lead chronically results in hypertension through the increased generation of reactive oxygen species; which in turn causes a decrease in NO accessibility (Vaziri and Ding, 2001).

1.2.2 Persistent organic pollutants

Persistent organic pollutants (POPs) are organic compounds that are toxic and non-degradable by the environment. They also present high volatility and low solubility in water, and are resistant to degradation whether abiotically or biotically (Velara et al., 2013). Thus, they bio accumulate in organisms and enter the food chain. Human exposure is through foods and environment. They induce a range of biological effects such as oxidative stress, endocrine system disruption, and alterations in enzymatic activities (Gascon et al. 2013). All of POPs tested in this study are different types of organohalides.

1.2.2.1 Polychlorinated biphenyls

Polychlorinated biphenyls or (PCBs) are synthetic organic molecules which are composed of 2 to 10 chlorine atoms attached to a biphenyl. They are colorless and odorless with 209 possible known congeners (Peters et al. 2014). They were manufactured to serve as coolants, plasticizers, and heat transfer fluids; however, they were banned in 1979 in the U.S. (Peter et al. 2014). PCBs are present in landfills, water supplies, and consumer products. They are able to release from these sites and products into air, making it an important source of exposure to humans (Carpenter, 2015). It has been believed that the primary source of human exposure is through ingestion of contaminated foods given that PCBs are fat soluble and bio accumulate in animal fat tissues (Carpenter, 2006).

PCBs are recognized as a human carcinogen, most notably malignant melanoma (Lauby-Secretan et al., 2013). Furthermore, recent epidemiological studies have found that PCB levels in serum are significantly linked to hypertension (Perkins et al. 2015). In a study, workers who are

exposed to PCBs displayed significant relationship between triglyceride levels and serum PCBs (Baker et al., 1980). Another study showed that PCBs serum levels was positively correlated with blood pressure and serum cholesterol levels (Kreiss et al., 1981). Certain PCBs are also associated with cardiovascular diseases as a study suggested that these PCBs are responsible for increased cholesterol and triglycerides by acting on aryl-hydrocarbon receptor that activates some P450 enzymes (Goncharov et al., 2007). Other studies have also shown that exposure to PCB increases the risk and incidence in the development of type 2 diabetes (Airaksinen et al. 2011). Another cohort study in women has shown a 67% increased risk of stroke for those who are exposed to PCBs from food consumption (Bergkvist et al. 2014).

1.2.2.2 Organochlorides

Organochlorides are organic compounds that contain a minimum of one chlorine atoms covalently bonded. They are mainly used as pesticides and in disinfectants. Ingestion of contaminated foods is an important root of exposure because, like PCBs, they are fat soluble and enter the food chain. Organochlorides in general cannot be metabolized, thus resulting in increase of concentration with increased exposure. Organochlorides include dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene p, p'-DDE (DDE), trans-nonachlor, oxychlordane, and pentachlorophenol. One study found that subjects who had diabetes exhibited higher blood levels of DDT and DDE (Morgan et al. 1980). Another study showed that DDE was significantly associated with diabetes in fishermen and their wives in Sweden (Rylander et al. 2005). Yet another study suggested that DDE plays a role in hypertension as it was positively associated with blood pressure (Velara et al., 2013; and

Henriquez-Hernandez et al., 2014). Chlordane has more than 140 isomers in which one of its metabolites is oxychlordane. Chlordane has also been shown to interfere with steroid receptor in animal studies (Cassidy et al., 1994), and have a strong association with type 2 diabetes (Lee et al., 2010). Exposure to pentachlorophenol has been suggested to increase the risk of cancer development (Cheng et al., 2014). Trans-nonachlor is another organochlorine molecule that has been found to predict the levels of triglycerides, and be in strong association with type 2 diabetes development from insulin resistance (Lee et al., 2011).

1.2.2.3 Toxaphene

A mixture of more than 650 chemicals (congeners), toxaphene had been mainly used as pesticide before its ban in the United States in 1990. Human exposure to toxaphene is through ingestion of contaminated foods, inhalation, and skin absorption. It enters the food chain as it bio accumulates in fat tissues of animals. Toxaphene has been identified as a carcinogen and a disruptor of the endocrine signaling system (Oostdam et al., 2005). Toxaphene has been shown to induce point mutations in bacteria, and leads to mutagenesis in *Salmonella typhimurium* in a dose-dependent fashion (Schrader et al., 1998).

1.2.2.4 Brominated flame retardants

Brominated flame retardants (BFR) are organobromine compounds that were mainly used as inhibitors of combustion and flame reducers. Due to their chemical properties, they are used mostly in electronics and cables. Polybrominated diphenyl ethers (PBDEs), a class of BFR, have been

used in production of furniture foams and carpets as well as in electronics. PBDEs are not linked covalently to the end products and are semi volatile; therefore, one source of human exposure is through the release of these compounds to the environment (Vuong et al., 2015). BFR are also able to bioaccumulate in fat tissues. It has been shown that brominated flame retardants disrupt the endocrine system, most notably decreasing thyroxin hormone in mammals (Dernerud, 2008).

1.2.2.5 Perfluorinated compounds

Perfluorinated compounds are organoflourine compounds and are able to reduce surface tension because of the many polar fluorine atoms found in these compounds. Perfluorooctane sulfonate (PFOS) are used in stain repellants, semiconductors, flame retardants, and aviation hydraulic fluids. Human exposure to PFOS have been through ingestion of contaminated foods and inhalation. A study found that pregnant rats treated with PFOS exhibited cell apoptosis and increased expression of p53 and cytochrome c in their hearts (Zeng et al., 2014). Another study found an association between PFOS serum levels and total cholesterol levels in a group of Danish population (Eriksen et al., 2013). PFOS has also been shown to increase serum insulin levels and decrease glucose tolerance in exposed rats during their developmental stages suggesting disturbance of glucose metabolism and development of insulin resistance (Lv et al., 2011).

1.3 Glucose metabolism

Every organism requires energy for survival. Energy is important for cells and organs to carry out their functions. Cells and organs use certain types of molecules to produce energy; in which adenosine triphosphate (ATP) is the main currency of energy. ATP can be produced by breaking

down carbohydrates, fat, or proteins; however, not all cells can use all forms. For example, the brain and red blood cells can only use a simple carbohydrate (glucose) to produce energy. The body stores excess glucose as glycogen mainly in liver for later breakdown when energy is needed and glucose is scarce. Other organisms use polymers of glucose as the base for their structural support as found in cellulose and chitin.

Glucose metabolism is a complex network of substrates, products, enzymes and coenzymes working to meet the cells' need of a desired outcome. In multi-step reactions known as glycolysis, the cells produce ATP and pyruvate by breaking down glucose. The cells can also do the opposite by producing glucose from other compounds in a process known as gluconeogenesis. Glycogen also undergoes synthesis and breakdown depending on the cells' need through processes known as glycogenesis and glycogenolysis, respectively.

Glucose metabolism overall is regulated by two hormones produced by the pancreas, insulin and glucagon. The pancreas releases insulin as a response to high levels of glucose in the blood stream, whereas glucagon is released when blood concentrations of glucose is low. Failure to regulate glucose levels in the body could result in metabolic diseases such as diabetes that could lead to further health complications such as cardiovascular diseases and loss of eye sight and or foot ulcers. Under certain conditions such as obesity, some cells do not respond to insulin in a condition called insulin resistance. Insulin becomes unable to promote muscle cells to uptake glucose and unable to inhibit glucose production in liver (Rondinone, 2006). Gluconeogenesis regulation is therefore important in modifying glucose levels. The development of insulin resistance is a primary identifier of diabetes in general, and more importantly in obesity (Zhao et al., 2015).

1.4 Cholesterol metabolism and lipoprotein signaling

Cholesterol is a sterol molecule that is found and synthesized in all animal cells. Diet is also another source of cholesterol and fats. Cholesterol is an important component of cell membranes as it helps maintain the structural integrity of the membrane. Cholesterol is also an important precursor for steroid hormones and vitamin D biosynthesis. The first and rate limiting step in the biosynthesis of steroid hormones is the cholesterol conversion to pregnenolone in the mitochondria by cytochrome p450 side chain cleavage enzyme (P450scc) (Hanukoglu, 1992). Cholesterol has been studied extensively due to its involvement in the regulation of cell function, its important role in maintaining membrane dynamics and structure, and its involvement in health complications and diseases.

Early studies have showed that cholesterol consumed through diet leads to an increase in cholesterol levels in the blood which in turn leads to arterial lesions (Willett, 2012). The most accepted hypothesis these days is that high consumption of cholesterol and saturated fats causes an increase in serum cholesterol levels that increase arterial plaques; in which the arteries become narrower over time resulting in a decreased blood flow to the heart and causing myocardial infraction (Willett, 2012; Shekelle & Stamler, 1989). Dietary cholesterol consumption increases low density lipoproteins (LDL) levels by supressing the synthesis of LDL receptors (Grundy et al., 1988), which in turn leads to increased cholesterol levels circulating in blood.

LDLs are one class of lipoproteins that are involved in cellular signaling through the transport of cholesterol throughout the organisms. Lipoproteins are macromolecules consisting of different lipids and apolipoproteins in a complex that transport lipids through blood (Wasan et al., 2008).

The main function of LDL is to transport cholesterol from liver to the body's cells, whereas high density lipoproteins (HDL) transport cholesterol from these cells to the liver. HDL is generally looked at as the healthy lipoproteins whereas LDL is looked at as the bad lipoproteins. HDL has been shown to have a protective effects on coronary heart disease (CHD), and LDL increases the risk of CHD (Gordon et al., 1977).

1.5 Obesity, diet, and diabetes

Obesity has long been known to be associated with increased risk of type 2 diabetes and other health complications. Risk of CHD, type 2 diabetes, and stroke increases with waist circumference increase (Despres, 2012). Insulin resistance and obesity result in an increase in fasting glucose levels that signifies greater risk of type 2 diabetes development; in that diabetes often develops after many years of insulin resistance development (Paneni et al., 2014). Environmental factors such as diet and lifestyle can also increase the risk of diabetes through insulin signaling disruptions, beta cells dysfunctions, and disruption of glucose transport (Kahn et al., 2006). Non-esterified fatty acids produced by adipose tissues to regulate metabolism induce beta cells dysfunction and insulin resistance; and are observed to be overproduced in obesity (Kahn et al., 2006). Overall, in general terms, increased lipolysis in adipocytes as a result of increased food intake and obesity impairs insulin secretion in the pancreas which causes increased gluconeogenesis in liver and decreased glucose uptake in muscles; ultimately leading to an increase in glucose plasma levels (Kahn et al., 2006).

Figure 1. Potential link between NCM exposure and metabolic and cardiovascular diseases (Dr. Dawn Jin Visual communication 2012-2013). Ingestion of Northern contaminants gets absorbed through the digestion system. As the liver is the detoxification system in the body, the contaminants could alter protein, lipid, steroid synthesis, and hormonal signaling in liver leading to disruption of glucose and cholesterol metabolism. These effects could promote insulin resistance and fatty liver as well as inflammation, endothelial dysfunction and oxidative stress. Ultimately, these effects lead to bigger health problems in kidney damage, hypertension, atherosclerosis, and the most feared heart strokes and heart attacks.

Table 1: The chemical composition of the Northern contaminant mixture (NCM) and concentrations used.

	Contaminants (% detected in Inuit plasma)	Chemical Dose		
		Mean levels detected in Inuit plasma samples (µg/L)	Low Dose (highest levels detected in Inuit plasma) (µg/L)	High Dose (µg/L)
Heavy Metals (3)	Cadmium (100%)	3.035	146.1	730.5
	Mercury (methylmercury) (100%)	10.997	241	1205
	Lead (100%)	39.368	497.3	2486.4
Total		53.4	884.4	4421.9
PCBs (12)	99 (98%)	0.17	4.7	23.5
	138 (100%)	0.534	19	95
	146 (97%)	0.18	6.1	30.5
	153 (100%)	1.333	40	200
	163 (98%)	0.221	6.2	31
	170 (99%)	0.216	6.18	30.9
	180 (100%)	0.813	22.7	113.5
	187 (100%)	0.287	9.1	45.5
	194 (95%)	0.182	5.1	25.5
	201 (97%)	0.167	4.7	23.5
	203 (92%)	0.105	2.5	12.5
Total		13.977	126.3	631.5
Organochlorine (4)	Oxychlorthane (100%)	0.431	16	80
	p,p'-DDE (100%)	3.232	50	250
	Trans-nonachlor (100%)	0.725	22	110
	Pentachlorophenol (100%)	0.914	18	90
Total		5.302	106	530
Toxaphene (1)	Parlar # 50	0.142	59.1	29.55
Total		0.142	59.1	29.55
Brominated flame retardants (2)	PBDE IUPAC #47 (55%)	0.072	2.4	12
	2,3,4,6-tetrabromophenol (63%)	0.036	1.39	6.97
Total		0.108	3.79	18.97
Perfluorinated compounds (1)	PFOS (100%)	29	470	2350
Total		29	470	2350
Total concentrations of contaminants		101.929	1596.4	7981.9

Table 2: Suggested northern contaminants concentrations limit; and their blood concentration in Canadian populations compared with Inuit.

	Inuit health survey (by Chan. 2007-2008)		
	Population guideline (µg/L)	Average Canadian population (µg/L)	Average Inuit concentration (µg/L)
mercury	20	< 2	25% > 20
cadmium	1	< 1	73% > 1
lead	100	< 100	< 100
PCBs	20	< 2	> 7
toxaphene	NA	< 0.04	> 0.16
DDT and DDE	NA	< 1	> 1
Chlordane	NA	< 0.1	> 0.9
PBDE	NA	< 0.1	< 0.1

Table 3: Average Inuit dietary intake.

Chan. L. 2007-2008 Inuit Health survey.		
Country food	Average intake (g/day)	Percent Inuit reported eating
Caribou meat	208	96
Arctic char	113	89
Dried caribou meat	125	75
Caribou tongue	15	71
Ringed seal liver	18	51
Beluga maktaaq	45	44
ringed seal meat	59	74

Chapter 2: Materials and methods

2.1 Animals and dosage

Liver tissues were extracted from JCR (LA)-Lepr obese (cp/cp) male rats and JCR (LA)-Lepr lean (+/cp, or +/+) male rats treated with a mixture of 23 inorganic and organic contaminants that were frequently detected at high concentrations in the Inuit blood samples. The cp/cp rats are homozygous for the autosomal recessive cp gene that results in Tyrosine763 Stop mutation for the leptin receptor (ObR). Without ObR, these rats become obese, hyperlipidemic, and hyperinsulinemic. Three dose levels were used; a) vehicle or 0 mg/kg, b) low dose or 1.6 mg/kg, and c) high dose or 8 mg/kg body weight (Table 1). These rats were also on two different diets, normal diet and high fat high sugar diet (Table 4). They are divided into two diets and three dose levels making a total of 12 different groups (Figure 2). Each animal is designated with a three letter code and a number (XXXn). The first X represents type of body and it could be L for lean or O for obese. The second X represents type of diet and it could be N for normal diet and H for high fat/sugar diet. The third X represents northern contaminant mixture dosage and it could be V for 0 mg/kg, L for 1.6 mg/kg and H for 8 mg/kg. The number at the end represents the batch number. The 12 groups are as follows: LNV (lean: normal diet: vehicle), LNL (lean: normal diet: low dose), LNH (lean: normal diet: high dose), LHV (lean: high fat/sugar diet: vehicle), LHL (lean: high fat/sugar diet: low dose), LHH (lean: high fat/sugar diet: high dose), ONV (obese: normal diet: vehicle), ONL (obese: normal diet: low dose), ONH (obese: normal diet: high dose), OHV (obese: high fat/sugar diet: vehicle), OHL (obese: high fat/sugar diet: low dose), and OHH (obese: high fat/sugar diet: high dose).

Figure 2: JCR rat treatment/ diet timeline. JCR rats obtained from University of Alberta were acclimatized to their new environment for two weeks. They were given two cookies each (AIN93G, New Brunswick, NJ) starting from week 3 to the end of week 5. From week 5 on, they were divided into two groups; normal diet that received the same AIN93G cookie, and high fat/sugar diet that received AIN93G modified with high fat/sugar. From week 7 to the end of week 11 (necroscopy), they were further divided into three NCM treatment groups (NCM dried on their cookies): vehicle (0 mg/kg/day NCM), low dose (1.6 mg/kg/day NCM), and high dose (8 mg/kg/day NCM). Rat tissues were collected at the end of the 11th week.

Table 4: The composition of the normal diet and high fat/sugar diet given to JCR rats.

Diet	Composition
Normal diet (AIN93G)	20% Casein 40% Starch 7% Soy oil 10% Sucrose
High fat/sugar diet (modified AIN93G)	10% Casein 9% Starch 13% Lard 7% Soy oil 40% Sucrose

2.2 RNA extraction:

mRNA from liver tissue was extracted using QIAGEN AllPrep DNA/RNA/Protein Mini Kit (Germantown, MD). 1 mL of RLT plus buffer + 10 % β -mercaptoethanol was added to 50-60 mg of pulverized tissue and homogenized by sonication for 20 seconds at 6°C using Covaris S2 Sonicator (Covaris, Woburn, Ma) at 20% cycle duty, 10 intensity, and 500 cycles per burst. The homogenized samples were then transferred to DNA spin columns where genomic DNA is eliminated. The flow through was transferred to RNA columns where proteins are eliminated. The RNA fraction was eluted in 30 μ l of RNase free water. The quality of the RNA was assessed by agarose gel electrophoresis and Nanodrop ND-1000 (Nanodrop Technologies, Inc./Thermo Scientific, Wilmington, DE) for concentration/contamination.

2.3 Reverse transcription and real time PCR:

One μ g of mRNA was reverse transcribed to cDNA using QIAGEN's RT2 First Strand Synthesis Kit (Germantown, MD). The mRNA was incubated with random primers, reverse transcriptase, and buffer for 15 minutes at 4°C then for 5 minutes at 95°C using Bio-Rad PCR Peltier Thermal Cycler (Bio-Rad, Hercules, CA).

RT2 SYBR Green ROX qPCR Mastermix from QIAGEN (Germantown, MD) was added to cDNA. Twenty five μ l of the mix were added to each well of the PCR preloaded plates with specific primers to target genes. Two kits from QIAGEN were used: a) RT2 Profiler PCR Array for Rat Lipoprotein and Cholesterol Metabolism (Cat. No. 330231 PARN-080ZA, Germantown,

MD) and b) RT2 Profiler PCR Array for Rat Glucose Metabolism (Cat. No. 330231 PARN-006ZA, Germantown, MD).

Real time PCR was done using Applied Biosystems 7500 Fast Real-Time PCR System with the following settings: 1 cycle for 10 minutes at 95°C, 40 cycles for 15 seconds and 1 minute at 95°C and 60°C, respectively.

2.4 Protein extraction:

Pulverized liver tissue (60-70 mg) was added to 1 mL of PBS + 1% phosphatase inhibitor and 1% protease inhibitor and homogenized using a Covaris S2 Sonicator for 1 minute at 20% duty cycle, 10 intensity, and 500 cycles/burst. The homogenate was centrifuged at 5000g for 5 minutes and 4°C. The supernatant was collected and the pellet/lipid layers were discarded. Protein concentrations were measured using Bio-Rad DC Assay (Bio-Rad, Hercules, CA).

2.5 ELISA:

Rat FBPase 2 ELISA Kit from Wuhan EIAab Science (Antibodies-Online.com; Cat. No. ABIN1133757), Rat PC ELISA Kit from Cusabio (Antibodies-Online.com; Cat. No. ABIN820002), Rat APOA1 ELISA Kit from Cusabio (Antibodies-Online.com; Cat. No. ABIN368046), and Rat SREBP-1C ELISA Kit from Cusabio (Antibodies-Online.com; Cat. No. ABIN858345) were used. Proteins from the previous step were diluted 3X in lysis buffer (PBS) for FBP2, 100X for PC and APOA1, and 50X for SREBF1. Protein extracts (100 µl) of it were added to the pre-coated plate with their corresponding specific antibody provided in the kits and

incubated overnight at 4°C. The liquid was removed and 100 µl of Detection Reagent A (Biotin linked antibody) were added to each well and incubated for 1.5 hrs at room temperature. The liquid was removed and the plate was washed 3 times using their Wash Buffer. Detection reagent B (Horseradish peroxidase linked antibody) (100 µl) was added to each well and incubated for 1.5 hrs at room temperature. The liquid was removed and the plate was washed 5 times with Wash Buffer. Ninety µl of substrate were added to each well and incubated for 20 minutes at room temperature. Fifty µL of Stop Solution were added to each well and the optical density of each well was determined using a microplate reader (BMG Labtech POLARstar OPTIMA; Ortenberg, Germany) set for 450 nm. ELISA results were normalized based on their corresponding protein concentration.

2.6 Statistical analyses:

Gene data (n of 6) analysis has been done using Qiagen template excel sheet (Germantown, MD) that uses student's t-test to compare differences between two groups. Outliers, c-q values, standard deviations, and melt curve checks were noted for each gene in order to eliminate as many false positives as possible. Protein data have been analyzed using IBM's SPSS 22 software (Armonk, NY). FBP2 and APOA1 data (both n of 5) have been analyzed using a randomized block design in order to eliminate day to day variations (One way ANOVA + day run as another predictor variable). PC (n of 5) and SREBF1 (n of 4) data have been analyzed using one way ANOVAs. Any test that results in a p-value of less than 0.05 is considered statistically significant.

Chapter 3: Results

3.1 Effects of NCM:

Following necropsy of the animals, serum levels of PCB 153 and 180 were measured (Table 5). These levels were in the range of the levels detected in Inuit plasma levels (Table 1).

3.1.1 NCM and glucose metabolism:

Reverse transcription real-time PCR profiler data (n of 6) shows varying degrees of up/downregulation for 82 genes that are involved in glucose metabolism. However, there are only a few significant changes as a response to NCM treatment (Appendix A). The NCM induces significant changes most notably to FBP2 and PC at the mRNA level. In order to determine if gene changes would translate into protein changes, ELISA was performed on both proteins. ELISA results show significant changes to FBP2 and PC. Both FBP2 and PC genes and proteins change significantly as a response to a high dose treatment; however, the low dose treatment would result in a significant change mostly at the mRNA level.

3.1.1.1 FBP2:

The NCM alters FBP2 gene and protein expression in JCR rat liver (Table 6). NCM increases the expression of FBP2 gene in lean rats (Table 6A). Lean rats treated with a high dose of NCM show significant upregulation in FBP2 gene compared to controls irrespective of their diet. Low dose treatments showed no significant effect on gene regulation when compared to their controls. FBP2 protein however, displays downregulation as a result of NCM treatment for lean rats (Figure 3). Lean rats treated with a high dose of NCM show significant decrease in protein expression

compared to their controls; also irrespective of their diet. The low dose treatment showed significant decrease only for high fat diet rats. The biggest significant increase in FBP2 mRNA is observed in lean, high fat diet, and high dose when compared to lean, high fat diet, and vehicle at a 146% increase ($p = 0.027$). The largest significant decrease in FBP2 protein is seen in the same group comparison at a 37% decrease ($p < 0.001$), from a mean concentration (n of 5) of 88.873 pg/mg to a 55.611 pg/mg.

Obese rats display significant upregulation of FBP2 gene across all NCM treatments regardless of their diet (Table 6B). There is also significant upregulation in FBP2 gene when the high dose is compared to the low dose. FBP2 protein is significantly under expressed as a response to the NCM treatment for all obese rats except for normal diet low dose when compared to its control (Figure 4). High dose to low dose comparisons display significant decrease in protein expression as well. The biggest significant increase in FBP2 mRNA for obese rats is observed in obese, normal diet, and high dose when compared to obese, normal diet, and vehicle at a 273% increase ($p = 0.017$). The same group comparison also displays the largest FBP2 protein decrease at a 47% decrease ($p = 0.007$), from a mean concentration (n of 5) of 133.8 pg/mg to 70.6 pg/mg.

3.1.1.2 PC:

The NCM treatments show a decrease in PC gene expression in lean rats (Table 7A). All NCM treatments have decreased the expression of PC gene significantly except for normal diet low dose when compared to their controls. The biggest decrease in PC mRNA is seen in lean, high fat diet, high dose when compared with lean, high fat diet, and vehicle at a 60% decrease ($p < 0.001$). NCM treatments also significantly decrease PC protein expression (Figure 5) in normal diet high dose

lean rats compared to its control. NCM treatments also exhibit significant decrease in PC protein expression in high fat diet rats across all dosages level. The largest significant decrease in PC protein is seen also in lean, high fat diet, and high dose when compared to lean, high fat diet, and low dose rats at a 70% decrease ($p = 0.03$), from a mean PC protein concentration (n of 5) of 4824 pg/mg to 1443 pg/mg.

Obese rats show significant decrease in PC gene and protein expression (Table 7B). Both PC gene and protein data (Figure 6) exhibit significant down regulation for high dose treatments when compared to their controls regardless of diet. The high fat diet obese rats show further significant under expression for high dose when compared with low dose in PC gene and protein. Obese, high fat diet, and high dose rats display the biggest decrease in both PC mRNA and protein compared to obese, high fat diet, and vehicle; in which, PC mRNA decreased 57% ($p = 0.002$); and PC protein decreased 71% ($p = 0.021$), from a mean PC protein concentration (n of 5) of 6902 pg/mg to 2029 pg/mg.

3.1.2 NCM and Cholesterol and Lipoprotein Metabolism

Reverse transcription real time- PCR profiler data shows varying degrees of up/ down regulation of 82 genes that are involved in the cholesterol metabolism and lipoprotein signaling (Appendix B). Of the 82 genes, only a few change significantly; namely APOA1 and SREBF1 at the mRNA level. ELISA results show some significant changes to the two proteins; however, mRNA and protein data for APOA1 and SREBF1 mostly do not overlap.

3.1.2.1 APOA1

APOA1 gene data (Table 8A) (n of 6) show significant under expression as a response to NCM low dose treatment compared to their controls irrespective of diet in lean rats. Normal diet rats treated with a high dose exhibits significant decrease in APOA1 gene expression when compared to control. APOA1 mRNA showed biggest decrease when lean, normal diet, low dose is compared to lean, normal diet, and vehicle at a 65% decrease ($p = 0.023$). APOA1 protein data for lean rats (Figure 7) show only one significant under expression; which is seen when comparing high dose to control in high fat diet lean rats at a 40% drop in protein concentration ($p = 0.014$), from a mean concentration (n of 5) of 34 $\mu\text{g}/\text{mg}$ to 20 $\mu\text{g}/\text{mg}$. All treatments compared to their controls (Figure 7) exhibit a reduction in protein concentration trend; however, most of them were not significantly different from controls.

Obese rats exhibits significant under expression of APOA1 gene only in the high fat diet group, when comparing high dose to its control (Table 8B), at a decrease of 61% ($p = 0.009$). At the protein level, APOA1 is significantly downregulated in high dose group compared to control regardless of diet; as well as significant decrease for high dose when compared to the low dose in high fat diet only (Figure 8). Obese, normal diet, and high dose rats display the most drop in APOA1 protein concentration when compared to obese, normal diet, and vehicle rats, at a reduction of 67% ($p = 0.032$) from a mean concentration (n of 5) of 52 $\mu\text{g}/\text{mg}$ to 30 $\mu\text{g}/\text{mg}$.

3.1.2.2 SREBF1

SREBF1 gene data for lean rats (Table 9A) show significant upregulation only for the high dose normal diet when compared to both control and the low dose normal diet. The biggest

SREBF1 is observed in lean, normal diet, and high dose rats compared to lean, normal diet, and vehicle rats at a 415% increase ($p = 0.009$). There were no significant difference in SREBF1 protein concentration between treatments in lean rats (Table 9B). SREBF1 protein data for lean rats (Figure 9) do not show any significant difference between treatments and control, even though a decreasing trend is observed.

Obese rat SREBF1 levels changed significantly when comparing the high dose to its control irrespective of their diet; and an added significance for a high dose compared to low dose in high fat diet ones (Figure 10). The largest decrease in SREBF1 protein is seen in obese, normal diet, and high dose rats compared to obese, normal diet, and vehicle at a 60% decrease ($p = 0.003$), from a mean SREBF1 concentration (n of 4) of 48679 pg/mg to 19595 pg/mg.

3.2 Effects of body weight and diet

Following necroscopy of the animals, body weights of all groups have been recorded (Table 5). Obese rats weigh 52.6% more than lean rats, from an average of 592.06 grams to 387.79 grams, respectively.

3.2.1 FBP2

Body weight has a significant effect on FBP2 gene and protein expression (Table 10A). FBP2 gene is significantly under expressed in obese rats compared to lean at a decrease of 49% ($p = 0.018$). FBP2 protein is significantly over expressed in obese compared to lean at an increase of 67% ($p = 0.002$), from a mean protein concentration (n of 5) of 132 pg/mg to 82 pg/mg (Figure 11). High fat diet however, did not contribute to any significant changes in either FBP2 mRNA or protein, a change of -29% ($p = 0.49$) and a change of 8% ($p = 0.548$), respectively.

3.2.2 PC

Body weight and diet did not have significant effects on PC mRNA expression (Table 10B). However, the expression of PC protein is significantly increased as a response to body weight and high fat diet (Figure 12). Body weight is responsible for a 262% increase in PC protein concentration ($p = 0.01$), from a mean protein concentration (n of 5) of 1934 pg/mg to 7006 pg/mg. An increase of 118% in PC protein concentration ($p = 0.047$) is observed as a response to high fat diet treatment (Table 10B).

3.2.3 APOA1

Body weight had a significant effect on APOA1 gene expression, but diet did not (Table 10C). APOA1 gene is significantly down regulated when obese rats are compared to lean (-31%, ($p = 0.33$)); however, there was no significant difference in APOA1 protein expression when obese rats are compared to lean (80% increase, ($p = 0.159$)) (Figure13). Diet had no effect on either APOA1 gene or protein levels; -32% $p = 0.235$, and 10% $p = 0.801$, respectively.

3.2.4 SREBF1

Body weight had a significant effect on SREBF1 gene and protein expression but not diet (Table 10D, Figure 14). SREBF1 mRNA is significantly overexpressed in obese rats compared to lean at a 982% increase ($p = 0.019$). SREBF1 protein is also significantly overexpressed at a 117% increase ($p = 0.006$), from a mean protein concentration (n of 4) of 22415 pg/mg to 48679 pg/mg.

High fat diet did not contribute to any significant changes in SREBF1 mRNA ($p = 0.218$) or protein ($p = 0.995$) levels.

Table 5: Average body weight of JCR rats; and average PCBs 153 and 180 levels in JCR rats sera.

Groups	Average body weight (grams)	Average sera PCB 153 (ug/L) (highest levels of PCB 153 detected in Inuit sera is 40 ug/L)	Average sera PCB 180 (ug/L) (highest levels of PCB 180 detected in Inuit sera is 22.7 ug/L)
LNV	393.89	0.00	0.00
LNL	385.94	16.29	11.44
LNH	389.08	18.70	12.61
LHV	386.96	0.62	0.00
LHL	386.88	12.82	9.61
LHH	384.01	17.69	13.16
ONV	625.19	0.00	0.00
ONL	601.82	12.80	9.94
ONH	585.74	16.01	13.67
OHV	596.15	0.12	0.03
OHL	565.30	6.54	5.29
OHH	578.15	10.69	8.32

Table 6: Effects of a NCM on FBP2 gene and protein expression in JCR lean (A) and obese (B) rat liver.

(A)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	LNV LNL	LNV LNH	LNL LNH	LHV LHL	LHV LHH	LHL LHH	
FBP2 mRNA (RT² PCR)	0.336	0.009	0.012	0.503	0.027	0.053	p- value*
	-20%	52%	62%	29%	146%	91%	% change
FBP2 PROTEIN (ELISA)	0.164	0.036	0.292	0.005	0.000	0.631	p- value**
	-22%	-33%	-14%	-34%	-37%	-5%	% change

(B)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	ONV ONL	ONV ONH	ONL ONH	OHV OHL	OHV OHH	OHL OHH	
FBP2 mRNA (RT² PCR)	0.019	0.017	0.026	0.001	0.000	0.000	p- value*
	125%	273%	134%	110%	199%	102%	% change
FBP2 PROTEIN (ELISA)	0.401	0.007	0.038	0.001	0.000	0.010	p- value**
	-12%	-47%	-40%	-27%	-46%	-27%	% change

*p-value represents the significance value from a student's t-test.

**p-value represents the significance value from a randomized block design.

Figure 3: Effects of NCM on FBP2 protein expression in JCR lean rat liver. (LNV) = lean normal diet vehicle= 0 mg/kg NCM. (LNL) = lean normal diet low dose= 1.6 mg/kg NCM. (LNH) = lean normal diet high dose= 8 mg/kg NCM. (LHV) = lean high fat diet vehicle= 0 mg/kg NCM. (LHL) = lean high fat diet low dose= 1.6 mg/kg NCM. (LHH) = lean high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). *[This figure compares: 1- LNV, LNL, and LNH with each other. 2- LHV, LHL, and LHH with each other. Results are based on normalized ELISA values and analyzed using a randomized block design].*

Figure 4: Effects of NCM on FBP2 protein expression in JCR Obese rat liver. (ONV) = obese normal diet vehicle= 0 mg/kg NCM. (ONL) = obese normal diet low dose= 1.6 mg/kg NCM. (ONH) = obese normal diet high dose= 8 mg/kg NCM. (OHV) = obese high fat diet vehicle= 0 mg/kg NCM. (OHL) = obese high fat diet low dose= 1.6 mg/kg NCM. (OHH) = obese high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). The symbol # represents significant difference within NCM treatments (between low dose and the high dose). *[This figure compares: 1- ONV, ONL, and ONH with each other. 2- OHV, OHL, and OHH with each other. Results are based on normalized ELISA values and analyzed using a randomized block design].*

Table 7: Effects of a NCM on PC gene and protein expression in JCR lean (A) and obese (B) rat liver.

(A)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	LNV LNL	LNV LNH	LNL LNH	LHV LHL	LHV LHH	LHL LHH	
PC mRNA (RT2 PCR)	0.526	0.038	0.023	0.000	0.000	0.050	p- value*
	-4%	-32%	-28%	-45%	-60%	-15%	% change
PC PROTEIN (ELISA)	0.237	0.013	0.132	0.041	0.030	0.044	p- value**
	-22%	-46%	-30%	-54%	-70%	-35%	% change

(B)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	ONV ONL	ONV ONH	ONL ONH	OHV OHL	OHV OHH	OHL OHH	
PC mRNA (RT2 PCR)	0.312	0.004	0.377	0.704	0.002	0.035	p- value*
	-15%	-50%	-30%	-8%	-57%	-37%	% change
PC PROTEIN (ELISA)	0.068	0.025	0.202	0.662	0.021	0.000	p- value**
	-32%	-68%	-53%	-11%	-71%	-67%	% change

*p-value represents the significance value from a student's t-test.

**p-value represents the significance value from one way ANOVAs.

Figure 5: Effects of NCM on PC protein expression in JCR lean rat liver. (LNV) = lean normal diet vehicle= 0 mg/kg NCM. (LNL) = lean normal diet low dose= 1.6 mg/kg NCM. (LNH) = lean normal diet high dose= 8 mg/kg NCM. (LHV) = lean high fat diet vehicle= 0 mg/kg NCM. (LHL) = lean high fat diet low dose= 1.6 mg/kg NCM. (LHH) = lean high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). The symbol # represents significant difference within NCM treatments (between low dose and the high dose). *[This figure compares: 1- LNV, LNL, and LNH with each other. 2- LHV, LHL, and LHH with each other. Results are based on normalized ELISA values and analyzed using one way ANOVAs].*

Figure 6: Effects of NCM on PC protein expression in JCR Obese rat liver. (ONV) = obese normal diet vehicle= 0 mg/kg NCM. (ONL) = obese normal diet low dose= 1.6 mg/kg NCM. (ONH) = obese normal diet high dose= 8 mg/kg NCM. (OHV) = obese high fat diet vehicle= 0 mg/kg NCM. (OHL) = obese high fat diet low dose= 1.6 mg/kg NCM. (OHH) = obese high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). The symbol # represents significant difference within NCM treatments (between low dose and the high dose). *[This figure compares: 1- ONV, ONL, and ONH with each other. 2- OHV, OHL, and OHH with each other. Results are based on normalized ELISA values and analyzed using one way ANOVAs].*

Table 8: Effects of a NCM on APOA1 gene and protein expression in JCR lean (A) and obese (B) rat liver.

(A)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	LNV LNL	LNV LNH	LNL LNH	LHV LHL	LHV LHH	LHL LHH	
APOA1 mRNA (RT2 PCR)	0.023	0.000	0.674	0.003	0.064	0.989	p- value*
	-65%	-61%	-3%	-62%	-56%	8%	% change
APOA1 PROTEIN (ELISA)	0.230	0.201	0.392	0.328	0.014	0.153	p- value**
	-30%	-47%	-30%	-17%	-40%	-27%	% change

(B)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	ONV ONL	ONV ONH	ONL ONH	OHV OHL	OHV OHH	OHL OHH	
APOA1 mRNA (RT2 PCR)	0.103	0.085	0.767	0.449	0.009	0.076	p- value*
	-58%	-72%	-33%	-26%	-61%	-50%	% change
APOA1 PROTEIN (ELISA)	0.161	0.032	0.238	0.785	0.012	0.014	p- value**
	-43%	-67%	-43%	7%	-52%	-55%	% change

*p-value represents the significance value from a student's t-test.

**p-value represents the significance value from a randomized block design.

Figure 7: Effects of NCM on APOA1 protein expression in JCR lean rat liver. (LNV) = lean normal diet vehicle= 0 mg/kg NCM. (LNL) = lean normal diet low dose= 1.6 mg/kg NCM. (LNH) = lean normal diet high dose= 8 mg/kg NCM. (LHV) = lean high fat diet vehicle= 0 mg/kg NCM. (LHL) = lean high fat diet low dose= 1.6 mg/kg NCM. (LHH) = lean high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). *[This figure compares: 1- LNV, LNL, and LNH with each other. 2- LHV, LHL, and LHH with each other. Results are based on normalized ELISA values and analyzed using a randomized block design].*

Figure 8: Effects of NCM on APOA1 protein expression in JCR Obese rat liver. (ONV) = obese normal diet vehicle= 0 mg/kg NCM. (ONL) = obese normal diet low dose= 1.6 mg/kg NCM. (ONH) = obese normal diet high dose= 8 mg/kg NCM. (OHV) = obese high fat diet vehicle= 0 mg/kg NCM. (OHL) = obese high fat diet low dose= 1.6 mg/kg NCM. (OHH) = obese high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls ($p < 0.05$). The symbol # represents significant difference within NCM treatments (between low dose and the high dose). *[This figure compares: 1- ONV, ONL, and ONH with each other. 2- OHV, OHL, and OHH with each other. Results are based on normalized ELISA values and analyzed using a randomized block design].*

Table 9: Effects of a NCM on SREBF1 gene and protein expression in JCR lean (A) and obese (B) rat liver.

(A)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	LNV LNL	LNV LNH	LNL LNH	LHV LHL	LHV LHH	LHL LHH	
SREBF1 mRNA (RT2 PCR)	0.314	0.009	0.040	0.528	0.246	0.116	p- value*
	54%	415%	204%	32%	174%	95%	% change
SREBF1 PROTEIN (ELISA)	0.147	0.072	0.950	0.261	0.116	0.741	p- value**
	-42%	-40%	3%	-31%	-42%	-16%	% change

(B)

	<i>Effects of mixture on LEAN and NORMAL DIET</i>			<i>Effects of mixture on LEAN and HIGH FAT DIET</i>			
	ONV ONL	ONV ONH	ONL ONH	OHV OHL	OHV OHH	OHL OHH	
SREBF1 mRNA (RT2 PCR)	0.434	0.078	0.249	0.306	0.400	0.652	p- value*
	-40%	-70%	-47%	-20%	9%	-14%	% change
SREBF1 PROTEIN (ELISA)	0.075	0.003	0.345	0.242	0.006	0.037	p- value**
	-42%	-60%	-30%	-15%	-59%	-52%	% change

*p-value represents the significance value from a student's t-test.

**p-value represents the significance value from one way ANOVAs.

Figure 9: Effects of NCM on SREBF1 protein expression in JCR lean rat liver. (LNV) = lean normal diet vehicle= 0 mg/kg NCM. (LNL) = lean normal diet low dose= 1.6 mg/kg NCM. (LNH) = lean normal diet high dose= 8 mg/kg NCM. (LHV) = lean high fat diet vehicle= 0 mg/kg NCM. (LHL) = lean high fat diet low dose= 1.6 mg/kg NCM. (LHH) = lean high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. *[This figure compares: 1- LNV, LNL, and LNH with each other. 2- LHV, LHL, and LHH with each other. Results are based on normalized ELISA values and analyzed using one way ANOVAs].*

Figure 10: Effects of NCM on SREBF1 protein expression in JCR Obese rat's liver. (ONV)

= obese normal diet vehicle= 0 mg/kg NCM. (ONL) = obese normal diet low dose= 1.6 mg/kg NCM. (ONH) = obese normal diet high dose= 8 mg/kg NCM. (OHV) = obese high fat diet vehicle= 0 mg/kg NCM. (OHL) = obese high fat diet low dose= 1.6 mg/kg NCM. (OHH) = obese high fat diet high dose= 8 mg/kg NCM. Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between NCM treatments (either low dose or high dose) and their corresponding controls) ($p < 0.05$). The symbol # represents significant difference within NCM treatments (between low dose and the high dose). *[This figure compares: 1- ONV, ONL, and ONH with each other. 2- OHV, OHL, and OHH with each other. Results are based on normalized ELISA values and analyzed using one way ANOVAs]*

Table 10: Effects of body weight and diet on the expression of genes and proteins: (A) FBP2, (B) PC, (C) APOA1, and (D) SREBF1 in JCR rat liver.

(A)

	<i>Effects of BODY WEIGHT</i>	<i>Effects of DIET</i>	
	LNV ONV	LNV LHV	
FBP2 mRNA (RT² PCR)	0.018	0.490	p- value*
	-49%	-29%	% change
FBP2 PROTEIN (ELISA)	0.002	0.548	p- value**
	67%	8%	% change

(B)

	<i>Effects of BODY WEIGHT</i>	<i>Effects of DIET</i>	
	LNV ONV	LNV LHV	
PC mRNA (RT² PCR)	0.318	0.199	p- value*
	27%	32%	% change
PC PROTEIN (ELISA)	0.010	0.047	p- value***
	262%	118%	% change

(C)

	<i>Effects of BODY WEIGHT</i>	<i>Effects of DIET</i>	
	LNV ONV	LNV LHV	
APOA1 mRNA (RT² PCR)	0.033	0.235	p- value*
	-31%	-32%	% change
APOA1 PROTEIN (ELISA)	0.159	0.801	p- value**
	80%	10%	% change

(D)

	<i>Effects of BODY WEIGHT</i>	<i>Effects of DIET</i>	
	LNV ONV	LNV LHV	
SREBF1 mRNA (RT² PCR)	0.019	0.218	p- value*
	982%	116%	% change
SREBF1 PROTEIN (ELISA)	0.006	0.955	p- value***
	117%	-1%	% change

*p-value represents the significance value from a student's t-test.

**p-value represents the significance value from a randomized block design.

***p-value represents the significance value from one way ANOVAs.

Figure 11: Effects of diet and body weight on FBP2 protein expression in JCR rat liver.

(LNV) = lean normal diet vehicle (0 mg/kg NCM). (LHV) = lean high fat diet vehicle (0 mg/kg NCM). (ONV) = obese normal diet vehicle (0 mg/kg NCM). Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between treatment and control. *[This figure compares treatment (LHV and ONV) to their control (LNV). Results are based on normalized ELISA values and analyzed using a randomized block design].*

Figure 12: Effects of diet and body weight on PC protein expression in JCR rat liver. (LNV)

= lean normal diet vehicle (0 mg/kg NCM). (LHV) = lean high fat diet vehicle (0 mg/kg NCM).

(ONV) = obese normal diet vehicle (0 mg/kg NCM). Data represents mean protein concentrations

$\pm$ 1 standard error. The symbol * represents significant difference between treatment and control.

[This figure compares treatment (LHV and ONV) to their control (LNV). Results are based on normalized ELISA values and analyzed using one way ANOVAs].

Figure 13: Effects of diet and body weight on APOA1 protein expression in JCR rat liver.

(LNV) = lean normal diet vehicle (0 mg/kg NCM). (LHV) = lean high fat diet vehicle (0 mg/kg NCM). (ONV) = obese normal diet vehicle (0 mg/kg NCM). Data represents mean protein concentrations $\pm$ 1 standard error. *[This figure compares treatment (LHV and ONV) to their control (LNV). Results are based on normalized ELISA values and analyzed using a randomized block design].*

Figure 14: Effects of diet and body weight on SREBF1 protein expression in JCR rat liver.

(LNV) = lean normal diet vehicle (0 mg/kg NCM). (LHV) = lean high fat diet vehicle (0 mg/kg NCM). (ONV) = obese normal diet vehicle (0 mg/kg NCM). Data represents mean protein concentrations $\pm$ 1 standard error. The symbol * represents significant difference between treatment and control. *[This figure compares treatment (LHV and ONV) to their control (LNV). Results are based on normalized ELISA values and analyzed using one way ANOVAs].*

Chapter 4: Discussion

Northern contaminants has been detected in Inuit's serum in which some of these contaminants are present in much higher concentrations than in the average Canadian population (Table 2) (Dewailley et al., 2006; and Chan et al, 2007-2008). In our study, JCR rats had been administered a much higher concentration of NCM per day (Table 1) than has been detected in blood of Inuit. It was necessary to use this much of NCM during a period of four weeks in order to establish NCM serum concentration in JCR rats comparable with that of Inuit at the end of the period (Mailloux et al., 2014) Results of serum PCB153 and 180 levels in our JCR rats following necroscopy can be seen in (Table 5); and they are in the range of Inuit levels detected (Table 1). Inuits exposure to these contaminants have been accumulated throughout their lifetime; whereas, we had to achieve those levels in four weeks. Acute exposure seen in this study is not fully representative of the chronic exposure seen in Inuit; however, it is as close as we can get to simulate the exposure with much realistic and affordable costs. Obese JCR rats used in this study are also not fully representative of human obesity state because this specific strain of rats develop obesity as a response to a mutation that takes place in leptin receptor (Mailloux et al., 2014), and are more prone to atherosclerosis, myocardial injury, insulin resistance, and disruption of glucose metabolism (Russel & Koeslag, 1990). Even though this mutation is not observed in humans, leptin resistance is observed and is believed to be associated with obesity (Mailloux et al., 2014). Therefore, obese JCR rats were studied to simulate the state of those with leptin resistance and disturbed hormonal balance, and lean rats simulate metabolically normal individuals.

In our study, we try to understand the effects of the 22 Northern contaminants as a whole on glucose and cholesterol metabolism, and lipoprotein signaling at the gene and protein level. Using gene profiler arrays, we have identified four genes (FBP2, PC, APOA1, and SREBF1) that change

significantly following NCM treatment amongst 164 genes profiled (Appendix A and B). We have observed a wide array of significant gene up/downregulation; however, these four genes are of primary interest as they either exhibited consistent change or are involved in important pathways in the development of diseases. FBP2 and PC are key regulators of gluconeogenesis, SREBF1 is an important transcription factor in lipogenesis, and APOA1 is an important fat transport molecule. Furthermore, the effects of high fat/sugar diet and body weight (i.e lean strain/obese strain) have been observed.

4.1 FBP2

Fructose 1,6-bisphosphatase (FBP2), isozyme 2 is an enzyme that converts fructose 1,6-bisphosphate to fructose 6-phosphate via hydrolysis. FBP2 is part of the glycolysis/gluconeogenesis pathway. It is also one of three enzymes that control gluconeogenesis in which they catalyze reactions that are irreversible (Bertinat et al. 2012). Fructose 2,6-bisphosphate competitively inhibits FBP2 and is considered a regulator of a key enzyme in glucose synthesis (Barthel and Schmoll, 2003). Therefore, excess fructose 2,6-bisphosphate levels that occur in the fed state is viewed as gluconeogenesis inhibitor through the binding to FBP2 and disabling its catalytic activity (Rider et al., 2004). Studies have shown that obese patients that have type 2 diabetes display increased endogenous glucose production that result from an increased FBP1/2 in liver (Nurjhan et al. 1992).

Fructose-1,6-bisphosphatase 2 (FBP2) gene results show a general trend of upregulation as a response to the mixture. NCM high dose treatments significantly overexpresses FBP2 mRNA when compared to their respective controls. Lean JCR rats that were on a high fat/sugar diet and treated with high dose display bigger significant change to their controls than the normal diet high

dose group compared to their controls (164% and 52%, respectively) (Table 3), suggesting that the high fat/sugar diet further intensifies the expression in combination with NCM treatment but not by itself. This is supported by the insignificant decrease in FBP2 mRNA expression as a response to high fat/sugar diet compared to normal diet (-27%).

The NCM treatment caused significant overexpression of FBP2 mRNA in obese JCR rats across all group comparisons, even amongst low dose and high dose treatments. The intensity of overexpression is also observed to be bigger compared to what lean rats display, suggesting that FBP2 mRNA expression in these leptin receptor corpulent rats is more sensitive to NCM than their lean counterparts. In a similar study using lean and obese JCR rats treated with NCM and ethanol instead of high fat/sugar diet, it has been shown that serum insulin levels in obese JCR rats are much higher than in lean rats (14.39 ± 1.44 ng/mL and 2.78 ± 0.36 ng/mL, respectively) (Mailloux et al., 2015). Given that insulin inhibits gluconeogenesis (Epstein, Moller & Flier, 1991), and FBP2 is a key enzyme that controls gluconeogenesis, it is expected that FBP2 mRNA expression would decrease in obese rats compared to lean rats in order to stop glucose synthesis. Indeed, in our study, we observe a significant under expression of FBP2 mRNA (-49%) in obese rats compared to lean rats; suggesting that insulin could be targeted by NCM. NCM in our lab's previous study has been shown to significantly decrease plasma insulin and glucagon levels indicative of pancreas function impairment (Mailloux et al., 2015). Thus, our theory is that NCM could impair pancreas function resulting in altered insulin levels that affects glucose metabolism in liver; most notably, affecting gluconeogenesis.

We observe however an interesting contrast in FBP2 protein expression. FBP2 protein expression seems to work in a negative feedback mechanism as any significant change in response to the NCM is in the opposite direction of its mRNA expression. Liver cells are producing excess

FBP2 mRNA as result of NCM treatment suggesting that liver cells could be sensing the need of producing glucose through insulin signaling; however, it is not translating into protein expression. This gives rise to the idea that FBP2 protein expression could be controlled by certain mechanisms; or that excess FBP2 mRNA causes its own protein degradation/ synthesis inhibition, and reduced mRNA expression might lead to over expression of the protein. The former is less likely as FBP2 is known to be ubiquitinated and degraded when glucose levels are high (Schork et al., 1995); and Mailloux et al., 2015 showed that glucose levels of lean and obese JCR rats treated with NCM did not change significantly. The latter could be explained by looking at the half-life of the mRNA. Some mRNAs display half-lives of several minutes while others might be as long as numerous days; in which the longer the mRNAs life, the slower it is to responding to stimuli (Yu & Russel, 2001). Therefore in theory, if FBP2 mRNA is long lived, only minimal amounts of it is required to produce more of the protein given that no inhibition is taking place. Other mechanisms could be involved as we do not have sufficient evidence to accept or reject any of these ideas. There could be something interfering with protein translation such as interfering RNA molecules (such as siRNA and miRNA), RNA-binding proteins whether at the 3' end or the 5' end, stress granules, and/or allosteric modifiers.

MicroRNAs (miRNA) are RNAs that are a part of a gene silencing complex; in which the complex is able to bind to an mRNA and inhibits protein synthesis and/or promote mRNA destabilization (Friedman et al., 2008). A study conducted by (Ramirez et al., 2013) have found that miRNA-33 significantly reduces protein levels of pyruvate carboxykinase and glucose 6 phosphatase; which are two of three important enzymes in gluconeogenesis (Bertinat et al., 2012). Given that FBP2 is the third important regulator of gluconeogenesis, it is possible that miRNA can target FBP2 and could explain FBP2 mRNA overexpression / FBP2 protein under expression by

inhibiting mRNA translation. However, it does not explain the opposite seen when we compare obese rats to lean rats. Obese rats show a decrease in FBP2 mRNA but an increase in FBP2 protein, suggesting that either something else is promoting mRNA translation and inhibiting the action of miRNA silencing complex if present; or it is just the result of a genetic difference between the strains. The latter is more fitting as JCR obese rats are greatly glucose intolerant (Russel and Koeslag, 1990), which would suggest that any minimal insignificant glucose level change would result in such a big and significant response. Overall, we speculate that altered insulin levels causes overexpression of FBP2 gene and gives rise to miRNA silencing complex that inhibits FBP2 protein synthesis; thereby inhibiting gluconeogenesis.

4.2 PC

Pyruvate carboxylase (PC) is another enzyme involved in glucose metabolism. PC enzyme is a biotin-dependant enzyme that catalyzes the conversion of pyruvate to oxaloacetate (Jitrapakdee et al., 2008) requiring the hydrolysis of one ATP molecule. The conversion of pyruvate to oxaloacetate is considered the first step in gluconeogenesis (Jitrapakdee et al., 2008). For tissues that are able to synthesize glucose and/or fat, pyruvate's fate is primarily determined by either pyruvate carboxylase or pyruvate dehydrogenase activities (either carboxylation or decarboxylation) (Davis-Van Thienen and Davis, 1981). An intermediate in the tricarboxylic acid cycle, oxaloacetate is further used in the synthesis of many molecules required for biosynthetic pathways (Jitrapakdee et al., 2008). PC is mainly localized in the mitochondrial matrix and is positively regulated by acetyl coenzyme A (Owen et al., 2002). PC's activity is high in liver (10-12 units/g of liver) (Owen et al., 2002), in which the levels of PC is further elevated during fasting

due to diminished glucose levels (Rothman et al., 1991). A study has shown that altered nutrition and diet (high sucrose and fat diet) in rats increases the expression of gluconeogenesis involved enzymes, namely liver PC (Bizeau et al., 2001).

Pyruvate carboxylase (PC), another important enzyme in gluconeogenesis that converts pyruvate to oxaloacetate, displays significant changes in mRNA and protein levels as a response to NCM treatment. The high dose treatment causes significant under expression of both PC mRNA and protein. High dose treatment response is always significant irrespective of diet and body weight; which suggests that NCM, at these levels, slows down the first committed step in gluconeogenesis. Significant reduction in PC mRNA and protein is also observed when comparing low dose to high dose animals; however, it is only noted for those rats that are also on high fat/sugar diet. Diet alone did have a significant reduction in PC protein but not mRNA, which indicates that there is no difference in transcription but the protein is either rapidly degraded or not fully degraded. Peroxisome proliferator-activated receptor- γ (PPAR γ) is a transcription factor which activates genes involved in lipogenesis and insulin regulation through the activation of PPAR-response element (Jitrapakdee et al., 2005). The same authors have found that PC levels (mRNA and protein) is correlated with PPAR γ levels and suggested that PC also has a role in lipogenesis (Jitrapakdee et al., 2005). They have also shown that PC gene contains PPRE sequences through transactivation studies and is highly conserved in mice and rats (Jitrapakdee et al., 2005). Animal studies have shown that PPAR γ ligands stop the development of atherosclerosis (Wang et al., 2011), and PPAR γ gene knockout would result in the rapid progression of atherosclerosis (Babaev, 2005). Our profiler arrays did not include PPAR γ gene; however, given its role in activating PC

transcription and our finding of decreased PC mRNA and protein expression, it is important to test PPAR γ levels as a response to NCM, body weight, and high fat/sugar diet.

4.3 APOA1

Apolipoprotein A1 (APOA1) is a member of apolipoprotein family that bind to lipids. APOA1 is synthesized in the liver and the intestine. Apolipoproteins' main function is to transport lipids throughout the body via the circulatory system. The main protein component of HDL is APOA1 that is responsible for the transport of cholesterol to the liver (Fielding & Fielding, 1981). Since high blood cholesterol levels is a risk factor for atherosclerosis development, and HDL is responsible for reverse cholesterol transport (inverse correlation), it is accepted that HDL displays anti-atherogenic effects (Mei & Atkinson, 2015). It does so by promoting the release of cholesterol from cholesterol filled macrophages in arterial walls and transporting it to the liver for breakdown or excretion (Barter & Rye, 1996). APOA1 is regulated by second messengers and hormones that include insulin, retinoids, and thyroid hormones (Haas et al., 2001). Decreased expression of APOA1 is seen in many other diseases. Decreased expression of APOA1 is linked to Alzheimer's disease (Lin et al., 2015). APOA1 levels in multiple sclerosis patients are lower than patients with no multiple sclerosis (Meyers et al., 2014). Lower levels of APOA1 is also associated with Parkinson's disease (Swanson et al., 2014).

NCM treatment resulted in some significant decrease in APOA1 mRNA and protein expression; however, only OHV/OHH group showed significant decrease in both mRNA and protein. One of the obstacles faced with APOA1 (and SREBF1) is that the standard deviation (SD) for the average

replicates is high in both mRNA and protein. For example, average SD for FBP2 and PC mRNA is less than 0.50 Cq values, whereas APOA1 is more than 0.80 Cq values. It can also be noted at the protein level as the error bars are relatively very big (Figure 7). Our cholesterol profiler array in general displays bigger SDs when compared to glucose profiler array; which suggests that either glucose metabolism is more tightly regulated or that rat to rat variations displays greater variability in cholesterol and lipoprotein gene expression. Since APOA1 is involved in cholesterol transport to the liver and excretion (Barter & Rye, 1996), it is empirical to add more replicas to our data in order to eliminate the variations within animals whether at the mRNA or protein levels. Our data as it is displays a general trend of decreased APOA1 protein expression. In our previous lab study, NCM treatment was shown to significantly increase total cholesterol in JCR rats (Mailloux et al., 2014); given that APOA1 transports cholesterol to the liver for breakdown/excretion (Barter & Rye, 1996), it is expected to observe a decrease in APOA1 protein level. APOA1 overexpression has been known to have a protective effects against atherosclerosis (Zhang et al., 2003); thus, under expression of APOA1 could accelerate atherosclerosis.

4.4 SREBF1 (SREBP-1c)

Sterol regulatory element-binding transcription factor 1 (SREBF1) is a protein of a family of transcription factors that binds to sterol regulatory element (SRE) and regulates the transcription of genes that synthesize cholesterol phospholipids, and fatty acids (Bengeochea-Alonso & Ericsson, 2007); however, this isoform specifically regulates lipogenesis genes (Horton, 2002). The inactive form of SREBF1 is found in the endoplasmic reticulum membrane; and as a response to low levels of sterols, it is cleaved and translocated to the nucleus where it binds to the (SRE)

(Horton, 2002). SREBF1 is regulated post transcriptionally by sterol induced suppression, and by other factors such as insulin, glucagon, and liver X activated receptors (LXR) (Horton, 2002). In transgenic mice, overexpression of the active form of SREBF1 in the liver shows a markedly increased fatty acid synthesis (Horton et al., 2002). SREBF1 controls cholesterol levels by regulating the expression of LDL receptor (Yokoyama et al., 1993). A study has shown that SREBF1 expression is higher in obese patients that have non-alcoholic fatty liver disease (NAFLD) when compared to lean controls (Pettinelli et al., 2009). Overexpression of SREBF1 is also associated with atherosclerosis, insulin resistance, and type 2 diabetes by inducing VLDL overproduction (Karasawa et al., 2011). In an insulin resistant mice study, inhibition of SREBF1 reduces accumulation of fats in the liver and reduces atherosclerosis (Li et al., 2011).

Sterol Regulatory Element Binding Transcription Factor 1 (SREBF1) mRNA data shows significant overexpression as a response to high dose NCM only in lean/normal diet rats. Like APOA1 data, SREBF1 data exhibits big SD at the mRNA level (SD of more than 1 Cq value). More replicates are necessary to see more significant differences and reduce rat to rat variations (the same applies to the protein). Our data indicates that there is no clear direct relationship between SREBF1 mRNA expression and SREBF1 protein expression since significant changes in mRNA expression as a response to NCM is only observed in lean rats, and significant changes in protein expression as a response to NCM is only observed in obese rats (Table 5). NCM treatment seems to overexpress SREBF1 mRNA in lean rats and under expresses it in obese rats (Table 5 A vs B). Obese JCR rats controls compared to lean JCR rats controls gives a 982% increase in SREBF1 mRNA ($p = 0.019$) and a 117% increase in SREBF1 protein ($p = 0.006$), which is consistent with (Elam et al., 2001)'s finding of overexpression in SREBF1 as a result of different genetic strain

rats (leptin receptor mutation). Furthermore, an increase in insulin is responsible for the increase in SREBF1 mRNA expression, and a decrease in insulin under expresses it (Owen et al., 2012), which also explains more of the big percent change, since our previous study showed an increase in serum insulin level in obese rats (Mailloux et al., 2014).

It has been shown that SREBF1 regulates and increases the activity of PPAR γ (Fajas et al., 1999) suggesting that SREBF1 is also responsible for indirect regulation of PC and gluconeogenesis. Therefore, it is important to look at PPAR γ to increase our understanding of the complex pathways studied here. SREBF1 is translated into an inactive protein bound to ER membrane in which it undergoes two proteolysis cleavages at the Golgi apparatus in order to translocate into the nucleus and activate the transcription of lipids by binding to SRE (Horton, 2002). We are unsure if the ELISA kit used is able to detect all forms; therefore, further testing needs to be conducted (such as Western blot) in order to understand which form of the protein is being targeted by NCM.

Chapter 5: Future directions and conclusion

5.1 Limitations and future directions

FBP2 mRNA levels are inversely related to its protein levels, and we suspect that an inhibition of translation is taking place by miRNA. miRNA testing is necessary to confirm the hypothesis. PC is correlated with PPAR γ , and SREBF1 regulates PPAR γ , it could be of interest to test PPAR γ levels that will add further understanding to the effects of NCM. APOA1 and SREBF1 data show big variations in mRNA and protein expression; therefore, additional replicas is important to reduce these variations. Another limitation in our study is that our ELISA kits are made to detect one form of our protein of interest. There might be post translational modifications such as phosphorylation or acetylation that disables us from detecting the protein when in fact it is present in a different form. Further testing needs to be done to confirm our results such as western blotting. Furthermore, the transcription factor SREBF1 is important in regulating lipogenesis and to some extent gluconeogenesis; therefore, downstream and upstream genes of SREBF1 action should be investigated.

5.2 Conclusion

NCM treatment induces alterations in glucose and cholesterol metabolism in JCR rats. JCR rats are a good animal model for the study of diabetes and heart disease among others due to their genetic background that makes them hyperinsulinemic and hyperlipidemic; conditions which correspond to that found in diabetics. We have identified four markers (FBP2, PC, APOA1, and

SREBF1) that play a role in lipid, cholesterol, and glucose metabolism in which NCM treatment induces significant changes to at the mRNA and/or protein levels. FBP2 and PC could aid us in understanding insulin resistance observed in JCR rats and Inuit better because they are key regulators of gluconeogenesis. APOA1 and SREBF1 could help us understand cholesterol metabolism better as APOA1 is important in the transport of cholesterol and SREBF1 is a transcription factor that regulates the expression of genes involved in lipogenesis. Future studies will focus on other enzymes in these pathways to determine whether these regulatory mechanisms reflect general changes in flux through these pathways in response to Northern contaminants.

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Appendix A: significant up/down regulation of 82 genes in glucose metabolism as a response to NCM Mixture, body weight, and diet.

GENE	LNV LNL		LNV LNH		LNL LNH		LHV LHL		LHV LHH		LHL LHH		LNV LHV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Acly	1.21	0.27	1.06	0.60	0.89	0.36	0.91	0.55	0.79	0.07	0.87	0.39	2.03	0.00
Aco1	0.93	0.57	1.22	0.13	1.33	0.06	0.88	0.04	1.24	0.07	1.41	0.03	1.17	0.19
Aco2	0.92	0.33	1.33	0.01	1.46	0.00	0.99	0.82	1.43	0.02	1.45	0.03	1.08	0.46
Ag1	0.89	0.35	1.02	0.83	1.16	0.14	0.91	0.30	0.97	0.62	1.07	0.67	1.03	0.73
Aldoa	1.00	0.90	1.14	0.27	1.16	0.24	1.01	0.95	1.42	0.03	1.41	0.14	0.93	0.55
Aldob	1.04	0.86	1.03	0.71	1.01	0.93	0.91	0.32	0.87	0.10	0.96	0.90	1.15	0.10
Aldoc	0.99	0.91	1.07	0.71	1.10	0.44	1.24	0.24	1.46	0.06	1.18	0.45	1.02	0.99
Bpgm	1.10	0.99	1.16	0.73	1.06	0.43	0.90	0.28	0.99	0.84	1.10	0.39	1.24	0.38
Cs	0.91	0.44	0.99	0.85	1.10	0.32	0.81	0.06	1.04	0.72	1.29	0.08	1.05	0.63
Dlat	0.88	0.41	1.04	0.58	1.19	0.15	0.96	0.54	1.13	0.28	1.17	0.14	1.07	0.18
Dld	0.87	0.27	1.00	0.84	1.72	0.09	1.10	0.81	1.32	0.11	1.21	0.07	0.91	0.62
Dlst	0.80	0.24	1.00	0.96	1.27	0.13	1.01	0.96	1.23	0.09	1.22	0.13	0.99	0.82
Eno1	0.82	0.16	0.98	0.74	1.21	0.06	0.85	0.11	0.87	0.34	1.02	0.95	1.13	0.39
Eno2	0.76	0.12	0.99	0.93	1.32	0.05	0.58	0.00	0.91	0.59	1.57	0.00	1.10	0.52
Eno3	1.02	0.80	1.05	0.78	1.04	0.80	0.79	0.20	0.94	0.75	1.20	0.37	0.97	0.79
Fbp1	0.86	0.33	1.08	0.43	1.27	0.06	0.81	0.03	0.81	0.03	1.00	0.94	1.05	0.52
Fbp2	0.82	0.34	1.56	0.01	1.67	0.01	1.29	0.50	2.46	0.03	1.91	0.05	0.79	0.49
Fh	0.98	0.87	1.20	0.11	1.24	0.08	1.17	0.21	1.41	0.00	1.21	0.38	0.89	0.26
G6pc	0.57	0.18	1.08	0.78	1.22	0.62	0.52	0.07	0.79	0.23	1.53	0.06	1.52	0.29
G6pc3	0.93	0.42	1.32	0.20	1.44	0.04	1.04	0.67	1.33	0.12	1.27	0.09	0.98	0.66
G6pd	0.88	0.36	0.79	0.08	0.91	0.21	0.64	0.00	0.69	0.02	1.09	0.32	1.09	0.25
Galm	0.92	0.55	0.86	0.28	0.94	0.52	0.77	0.01	0.77	0.04	0.99	0.87	1.02	0.93
Gapdh	0.89	0.64	1.08	0.38	1.23	0.16	0.93	0.60	1.16	0.34	1.26	0.35	1.17	0.15
Gapdhs	0.97	0.86	0.81	0.64	0.85	0.75	1.02	0.90	1.13	0.63	1.10	0.69	0.84	0.46
Gck	0.86	0.76	0.80	0.49	0.87	0.56	0.50	0.06	0.50	0.10	0.99	0.84	1.36	0.23
Gpi	1.01	0.96	1.02	0.81	1.03	0.93	1.05	0.46	1.15	0.14	1.10	0.43	1.05	0.64
Gsk3a	0.86	0.22	0.94	0.60	1.10	0.24	0.79	0.01	0.94	0.79	1.19	0.03	1.01	0.85
Gsk3b	0.95	0.57	0.96	0.63	1.03	0.71	0.87	0.09	0.98	0.84	1.13	0.34	0.94	0.41
Gys1	1.01	0.97	1.12	0.48	1.13	0.38	0.87	0.27	0.95	0.76	1.10	0.56	1.09	0.75
Gys2	1.26	0.27	0.94	0.64	0.75	0.21	0.84	0.24	0.82	0.02	0.98	0.78	1.25	0.15
H6pd	0.85	0.48	0.99	0.91	1.18	0.45	0.88	0.11	0.94	0.56	1.08	0.65	1.13	0.29
Hk2	1.06	0.78	1.06	0.73	1.01	0.85	0.74	0.13	0.85	0.57	1.15	0.45	1.09	0.76
Hk3	1.22	0.34	0.74	0.22	0.62	0.07	0.71	0.09	0.66	0.23	0.93	0.92	1.16	0.30
Idh1	1.01	0.85	1.05	0.44	1.05	0.58	1.03	0.88	1.12	0.29	1.09	0.58	1.05	0.44
Idh2	0.93	0.47	0.69	0.05	0.75	0.01	0.78	0.01	0.79	0.04	1.01	1.00	1.31	0.02
Idh3a	0.85	0.05	1.23	0.07	1.47	0.00	1.00	0.91	1.40	0.03	1.40	0.01	0.99	0.74
Idh3b	0.87	0.24	1.12	0.20	1.31	0.02	0.99	0.85	1.17	0.06	1.18	0.05	1.00	0.98
Idh3g	0.92	0.29	1.13	0.02	1.25	0.01	0.85	0.00	1.05	0.40	1.24	0.09	1.17	0.02
Mdh1	0.99	0.85	1.09	0.26	1.11	0.20	0.83	0.02	0.91	0.11	1.10	0.42	1.25	0.08
Mdh1b	0.89	0.88	0.99	0.84	1.13	0.73	0.68	0.21	0.73	0.25	1.08	0.59	1.40	0.21
Mdh2	1.02	0.83	1.05	0.43	1.04	0.53	0.91	0.26	1.04	0.53	1.15	0.44	1.16	0.06
Ogdhl	0.83	0.48	0.72	0.02	0.88	0.35	0.71	0.03	0.64	0.02	0.90	0.46	0.99	0.95
Pc	0.96	0.53	0.59	0.04	0.72	0.02	0.55	0.00	0.43	0.00	0.77	0.05	1.33	0.20
Pck1	1.15	0.90	0.87	0.42	0.77	0.16	0.65	0.01	0.73	0.02	1.13	0.37	1.22	0.46
Pck2	0.98	0.84	1.05	0.69	1.09	0.32	0.92	0.48	1.02	0.82	1.11	0.67	1.54	0.04
Pdha2	1.03	0.75	1.00	0.83	0.98	0.67	0.97	0.89	0.97	0.71	1.00	0.81	1.20	0.26
Pdhh	0.95	0.60	1.05	0.78	1.12	0.29	0.90	0.15	1.02	0.75	1.13	0.18	0.99	0.89
Pdhx	0.90	0.24	1.08	0.06	1.22	0.01	0.92	0.23	1.15	0.14	1.25	0.07	1.01	0.86
Pdk1	0.99	0.91	1.03	0.68	1.05	0.66	0.84	0.01	1.00	0.89	1.19	0.35	1.12	0.11
Pdk2	1.06	0.59	1.20	0.02	1.15	0.28	1.17	0.09	1.21	0.01	1.04	0.93	1.02	0.85
Pdk3	1.07	0.60	0.80	0.17	0.75	0.09	0.80	0.36	0.87	0.53	1.10	0.70	0.98	0.77
Pdk4	0.78	0.53	0.98	0.93	1.28	0.38	0.39	0.03	1.21	0.61	3.09	0.00	1.12	0.46
Pdp2	1.06	0.97	0.88	0.27	0.84	0.11	0.73	0.01	0.67	0.01	0.92	0.36	1.29	0.05
Pdpr	0.91	0.35	1.04	0.52	1.15	0.11	0.88	0.10	1.01	0.85	1.15	0.20	0.99	0.90
Pfkl	0.84	0.19	0.96	0.66	1.16	0.13	0.88	0.12	1.01	0.79	1.15	0.05	1.03	0.85

GENE	LNV LNL		LNV LNH		LNL LNH		LHV LHL		LHV LHH		LHL LHH		LNV LHV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Pgam2	1.16	0.69	0.98	0.68	0.85	0.20	0.75	0.08	0.97	0.84	1.31	0.06	1.28	0.30
Pgk1	0.86	0.11	1.17	0.03	1.38	0.00	0.88	0.29	1.31	0.02	1.50	0.06	1.04	0.70
Pgk2	0.94	0.58	1.04	0.91	1.12	0.50	0.81	0.48	1.02	0.96	1.26	0.50	1.07	0.76
Pgls	0.93	0.56	1.03	0.81	1.13	0.21	0.74	0.00	0.82	0.06	1.11	0.35	1.20	0.09
Pgm1	0.89	0.44	0.95	0.68	1.08	0.59	0.90	0.25	0.90	0.46	1.01	0.86	1.09	0.50
Pgm2	0.79	0.17	1.11	0.28	1.43	0.03	1.16	0.31	1.49	0.00	1.29	0.14	0.88	0.28
Pgm3	1.04	0.90	0.94	0.53	0.91	0.53	0.96	0.55	0.98	0.73	1.02	0.79	1.16	0.02
Phka1	0.88	0.25	0.85	0.21	0.99	0.93	0.75	0.01	0.80	0.07	1.07	0.65	1.08	0.53
Phkb	1.21	0.25	1.11	0.48	0.93	0.56	0.84	0.22	1.00	0.76	1.19	0.24	1.27	0.23
Phkg1	1.27	0.27	0.76	0.12	0.61	0.07	0.75	0.29	0.77	0.23	1.03	0.98	0.96	0.92
Phkg2	0.88	0.29	0.97	0.71	1.11	0.19	0.82	0.06	0.85	0.36	1.04	0.79	1.04	0.82
Pklr	1.32	0.35	0.81	0.40	0.62	0.04	0.77	0.20	0.57	0.01	0.74	0.39	1.44	0.15
Prps1	0.89	0.23	1.00	0.98	1.14	0.13	0.92	0.30	1.11	0.21	1.22	0.26	0.99	0.92
Prps1l1	1.02	0.86	1.06	0.91	1.06	0.90	0.99	0.95	1.51	0.16	1.53	0.13	1.11	0.74
Pygl	0.90	0.45	0.80	0.19	0.90	0.61	0.79	0.06	0.67	0.05	0.86	0.19	1.05	0.59
Pygm	1.09	0.45	0.91	0.32	0.84	0.15	0.68	0.07	0.80	0.23	1.19	0.53	1.22	0.34
Rbks	0.92	0.36	0.98	0.80	1.08	0.36	0.80	0.01	0.93	0.18	1.16	0.27	1.08	0.41
Rpia	0.85	0.13	1.12	0.17	1.35	0.00	0.93	0.45	1.26	0.03	1.36	0.02	0.91	0.30
Sdha	0.93	0.53	1.21	0.13	1.32	0.07	1.05	0.55	1.24	0.06	1.18	0.04	0.93	0.54
Sdhb	0.91	0.27	1.27	0.00	1.42	0.00	1.00	0.95	1.35	0.00	1.36	0.16	0.95	0.54
Sdhc	0.81	0.19	1.04	0.77	1.30	0.08	0.90	0.26	1.03	0.69	1.15	0.02	0.98	0.93
Sdhd	0.91	0.35	1.08	0.39	1.20	0.05	0.87	0.00	1.00	0.97	1.15	0.24	1.07	0.40
Suc1a2	0.94	0.56	1.07	0.40	1.15	0.04	0.86	0.04	1.05	0.43	1.23	0.11	0.99	0.94
Suc1g1	0.83	0.08	1.11	0.17	1.36	0.02	0.92	0.12	1.44	0.01	1.58	0.02	0.97	0.77
Suc1g2	0.93	0.66	1.32	0.02	1.43	0.02	1.00	0.94	1.28	0.03	1.28	0.01	1.06	0.66
Taldo1	1.00	0.98	1.11	0.20	1.12	0.20	0.90	0.31	1.07	0.40	1.19	0.30	1.06	0.43
Tkt	0.96	0.76	1.15	0.14	1.21	0.12	0.87	0.15	1.03	0.75	1.19	0.29	1.37	0.05
Tpi1	0.91	0.42	0.97	0.74	1.09	0.35	0.90	0.31	1.09	0.35	1.21	0.35	1.01	0.95
Ugp2	1.19	0.26	0.65	0.01	0.55	0.00	0.84	0.37	0.66	0.10	0.78	0.42	1.11	0.39
Actb	0.99	0.89	0.86	0.01	0.89	0.07	0.85	0.08	0.90	0.38	1.07	0.47	1.09	0.36
B2m	1.00	0.96	1.00	0.89	1.01	0.77	0.82	0.04	0.89	0.26	1.10	0.51	0.97	0.56
Hprt1	1.00	0.96	1.23	0.14	1.25	0.18	1.11	0.44	1.65	0.00	1.49	0.14	1.00	0.99
Ldha	0.79	0.38	0.70	0.04	0.90	0.34	0.54	0.00	0.53	0.00	0.99	0.90	1.16	0.32
Rplp1	0.90	0.44	1.20	0.01	1.35	0.01	0.89	0.19	1.12	0.15	1.26	0.29	1.07	0.35

GENE	ONV ONL		ONV ONH		ONL ONH		OHV OHL		OHV OHH		OHL OHH		LNV ONV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Acly	0.59	0.14	0.66	0.14	1.12	0.61	1.10	0.41	0.66	0.12	0.85	0.63	5.25	0.02
Aco1	1.23	0.04	1.70	0.01	1.39	0.01	1.56	0.00	1.27	0.21	1.16	0.36	0.87	0.13
Aco2	1.15	0.36	1.37	0.08	1.20	0.33	1.62	0.01	0.96	0.70	0.84	0.19	1.06	0.56
Agl	1.15	0.16	1.07	0.50	0.93	0.44	1.63	0.00	1.18	0.33	1.03	0.73	0.75	0.01
Aldoa	1.29	0.32	1.37	0.03	1.06	0.80	1.48	0.02	1.39	0.01	1.32	0.07	0.88	0.29
Aldob	0.90	0.12	0.77	0.01	0.86	0.05	1.24	0.02	0.69	0.00	0.79	0.04	1.41	0.00
Aldoc	1.44	0.00	1.59	0.01	1.10	0.41	0.98	0.48	1.14	0.47	1.65	0.45	0.61	0.02
Bpgm	1.18	0.21	1.16	0.20	0.99	0.93	1.09	0.45	0.97	0.66	1.26	0.49	0.88	0.29
Cs	1.16	0.22	1.01	0.91	0.88	0.37	1.43	0.05	1.05	0.88	1.04	0.89	0.92	0.42
Dlat	0.86	0.23	0.87	0.24	1.01	0.95	1.12	0.38	0.91	0.46	1.15	0.96	1.48	0.00
Dld	1.05	0.50	1.16	0.16	1.10	0.33	1.58	0.01	1.07	0.64	0.95	0.67	0.79	0.01
Dlst	1.22	0.17	1.31	0.08	1.08	0.50	1.72	0.03	1.10	0.71	0.91	0.52	0.83	0.05
Eno1	0.94	0.62	0.92	0.50	0.99	0.92	1.34	0.08	0.82	0.31	0.86	0.44	1.37	0.04
Eno2	1.11	0.59	1.01	0.94	0.91	0.53	1.24	0.43	0.77	0.23	0.88	0.38	0.74	0.13
Eno3	1.98	0.34	0.96	0.63	0.49	0.34	1.17	0.53	1.00	0.82	1.21	0.16	0.96	0.68
Fbp1	1.03	0.70	0.93	0.63	0.90	0.44	1.40	0.02	0.77	0.05	0.79	0.07	0.73	0.00
Fbp2	2.25	0.02	3.73	0.02	2.34	0.03	2.10	0.00	2.99	0.00	2.02	0.00	0.51	0.02
Fh	1.11	0.47	1.19	0.31	1.08	0.51	1.44	0.04	0.97	0.65	0.95	0.56	0.74	0.08
G6pc	0.88	0.75	0.68	0.31	0.78	0.63	0.77	0.45	0.47	0.01	0.86	0.35	0.76	0.21

GENE	ONV ONL		ONV ONH		ONL ONH		OHV OHL		OHV OHH		OHL OHH		LNV ONV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
G6pc3	1.14	0.56	1.24	0.27	1.09	0.50	1.66	0.06	1.27	0.43	1.08	0.79	0.72	0.07
G6pd	0.72	0.11	0.76	0.21	1.06	0.52	1.35	0.24	1.14	0.75	1.20	0.28	1.41	0.04
Galm	1.11	0.60	1.07	0.71	0.97	0.80	1.30	0.27	0.84	0.21	0.91	0.47	0.59	0.00
Gapdh	0.92	0.30	0.98	0.90	1.07	0.50	1.30	0.07	0.89	0.20	0.97	0.65	1.93	0.00
Gapdhs	1.11	0.49	0.85	0.66	0.77	0.25	1.37	0.18	0.93	0.73	0.97	0.87	1.23	0.42
Gck	0.93	0.69	0.17	0.18	0.18	0.23	0.64	0.12	0.37	0.08	0.82	0.94	7.80	0.00
Gpi	0.87	0.33	0.95	0.71	1.09	0.47	1.42	0.00	0.98	0.91	0.97	0.91	1.58	0.00
Gsk3a	1.04	0.85	0.25	0.30	0.24	0.14	1.47	0.03	1.07	0.65	1.03	0.84	0.77	0.05
Gsk3b	0.96	0.37	0.98	0.76	1.02	0.69	1.42	0.03	1.05	0.88	1.04	0.77	0.96	0.51
Gys1	1.40	0.31	1.03	0.93	0.74	0.34	1.11	0.39	0.88	0.41	1.12	0.61	0.85	0.43
Gys2	0.95	0.54	1.10	0.51	1.16	0.20	1.72	0.00	1.13	0.29	0.93	0.70	1.26	0.18
H6pd	1.20	0.43	1.09	0.71	0.91	0.58	1.25	0.31	0.84	0.39	0.95	0.68	0.79	0.15
Hk2	1.11	0.44	0.90	0.98	0.81	0.42	1.33	0.08	1.07	0.62	1.13	0.64	0.73	0.09
Hk3	1.07	0.68	0.80	0.16	0.75	0.06	1.48	0.16	1.12	0.70	1.07	0.99	0.52	0.00
Idh1	1.14	0.21	1.32	0.07	1.17	0.06	1.51	0.02	1.13	0.38	1.06	0.72	0.68	0.00
Idh2	1.28	0.16	1.06	0.72	0.83	0.17	1.34	0.14	0.80	0.18	0.84	0.30	0.97	0.77
Idh3a	1.16	0.23	1.11	0.40	0.96	0.73	1.57	0.02	1.05	0.87	0.95	0.47	0.99	0.76
Idh3b	1.11	0.46	1.21	0.10	1.10	0.36	1.58	0.02	1.12	0.48	1.00	0.97	0.77	0.01
Idh3g	1.14	0.07	1.09	0.28	0.96	0.52	1.39	0.04	0.93	0.46	0.95	0.56	1.13	0.03
Mdh1	0.92	0.27	0.90	0.38	0.98	0.96	1.14	0.28	0.69	0.01	0.86	0.19	1.56	0.00
Mdh1b	0.97	0.95	1.08	0.59	1.11	0.62	1.10	0.80	1.20	0.77	1.55	0.13	0.95	0.84
Mdh2	1.04	0.42	1.09	0.33	1.05	0.44	1.33	0.02	0.95	0.28	1.01	0.80	1.03	0.61
Ogdhl	1.02	0.85	1.05	0.64	1.03	0.69	1.03	0.93	0.94	0.60	1.30	0.04	0.34	0.00
Pc	0.85	0.31	0.50	0.00	0.70	0.38	0.92	0.70	0.43	0.00	0.63	0.03	1.27	0.32
Pck1	0.99	0.99	0.56	0.11	0.57	0.16	0.92	0.87	0.45	0.00	0.69	0.18	0.70	0.11
Pck2	1.13	0.26	1.17	0.44	1.03	0.60	1.47	0.07	0.87	0.12	0.84	0.26	1.14	0.38
Pdha2	1.01	0.95	1.13	0.49	1.12	0.45	0.96	0.99	1.04	0.82	1.54	0.08	1.01	1.00
Pdhb	0.91	0.43	0.87	0.26	0.96	0.72	1.41	0.07	0.87	0.30	0.87	0.38	1.34	0.05
Pdhx	1.09	0.13	1.19	0.07	1.09	0.14	1.56	0.01	1.09	0.42	0.99	0.82	0.91	0.21
Pdk1	0.92	0.33	0.91	0.28	0.99	0.99	1.19	0.20	0.78	0.07	0.93	0.56	1.64	0.00
Pdk2	1.01	0.98	1.04	0.66	1.03	0.69	1.45	0.01	0.96	0.66	0.94	0.54	1.34	0.00
Pdk3	0.92	0.45	0.93	0.95	1.02	0.75	1.30	0.03	1.00	0.80	1.09	0.47	0.83	0.26
Pdk4	1.13	0.46	1.92	0.06	1.70	0.09	2.01	0.04	1.89	0.03	1.33	0.23	0.58	0.02
Pdp2	0.78	0.01	0.85	0.26	1.09	0.46	1.31	0.10	0.78	0.20	0.84	0.34	0.70	0.00
Pdpr	1.16	0.12	1.17	0.13	1.02	0.90	1.57	0.03	1.10	0.59	0.99	0.91	0.88	0.08
Pfkl	1.05	0.65	1.08	0.46	1.03	0.77	1.44	0.09	0.98	0.76	0.97	0.71	0.85	0.11
Pgam2	2.11	0.34	1.09	0.66	0.52	0.34	1.44	0.07	1.20	0.41	1.18	0.37	1.04	0.96
Pgk1	1.07	0.22	1.10	0.31	1.04	0.38	1.34	0.03	0.96	0.44	1.01	0.87	1.12	0.28
Pgk2	1.28	0.51	0.96	0.73	0.75	0.22	0.94	0.69	0.88	0.96	1.33	0.26	0.80	0.42
Pgls	1.06	0.18	1.08	0.14	1.03	0.63	1.31	0.04	0.82	0.03	0.88	0.12	0.87	0.08
Pgm1	1.12	0.51	1.10	0.47	0.99	0.99	1.47	0.10	0.93	0.77	0.90	0.61	1.01	1.00
Pgm2	1.09	0.25	1.35	0.06	1.24	0.09	1.71	0.00	1.46	0.00	1.21	0.01	0.79	0.09
Pgm3	1.05	0.78	1.30	0.07	1.24	0.18	1.57	0.00	1.16	0.27	1.05	0.73	0.54	0.00
Phka1	0.99	0.69	0.85	0.48	0.85	0.54	1.07	0.78	0.63	0.05	0.84	0.24	0.94	0.54
Phkb	1.03	0.68	0.92	0.65	0.90	0.50	1.32	0.24	1.10	0.82	1.18	0.45	1.20	0.34
Phkg1	1.73	0.30	1.12	0.55	0.64	0.33	0.96	0.67	0.76	0.24	1.13	0.55	0.73	0.06
Phkg2	1.07	0.43	1.03	0.54	0.97	0.72	1.34	0.08	0.91	0.45	0.96	0.66	0.75	0.01
Pklr	0.67	0.09	0.52	0.04	0.78	0.62	1.04	0.80	0.51	0.01	0.70	0.04	5.33	0.00
Prps1	1.07	0.46	1.16	0.20	1.09	0.21	1.49	0.01	1.08	0.42	1.03	0.82	0.58	0.00
Prps1l1	1.42	0.17	1.70	0.11	1.20	0.56	1.23	0.28	1.41	0.24	1.62	0.18	0.91	0.48
Pygl	1.03	0.78	0.80	0.06	0.78	0.05	1.06	0.75	0.68	0.04	0.91	0.50	1.00	0.84
Pygm	2.63	0.34	1.02	0.76	0.39	0.34	1.27	0.23	1.16	0.51	1.29	0.19	0.61	0.02
Rbks	1.04	0.61	1.00	0.97	0.97	0.81	1.38	0.04	0.92	0.43	0.94	0.60	0.96	0.55
Rpia	1.00	0.96	1.05	0.71	1.05	0.74	1.66	0.01	1.19	0.29	1.02	0.99	1.20	0.10
Sdha	1.09	0.14	1.09	0.28	1.00	0.94	1.59	0.00	1.15	0.25	1.02	0.88	0.90	0.23
Sdhb	1.13	0.03	1.22	0.08	1.08	0.12	1.51	0.00	1.11	0.17	1.04	0.57	1.00	0.93
Sdhc	1.11	0.62	1.16	0.41	1.05	0.70	1.61	0.03	1.04	0.87	0.91	0.59	0.79	0.11
Sdhd	1.11	0.36	1.04	0.72	0.94	0.25	1.37	0.06	0.86	0.22	0.89	0.27	0.91	0.19
Suc1a2	1.13	0.07	1.08	0.33	0.96	0.58	1.42	0.04	0.92	0.46	0.92	0.45	0.79	0.01
Suc1g1	1.16	0.25	1.45	0.04	1.25	0.03	1.52	0.03	1.25	0.21	1.17	0.30	0.75	0.01
Suc1g2	1.18	0.41	1.36	0.10	1.15	0.12	1.49	0.06	1.07	0.72	1.01	0.95	0.99	0.99
Taldo1	0.99	0.98	0.96	0.64	0.97	0.66	1.33	0.05	0.92	0.08	0.98	0.57	1.36	0.01

GENE	ONV ONL		ONV ONH		ONL ONH		OHV OHL		OHV OHH		OHL OHH		LNV ONV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Tkt	0.80	0.07	1.00	0.97	1.26	0.15	1.26	0.03	0.94	0.81	1.06	0.57	1.52	0.01
Tpi1	1.08	0.36	1.15	0.19	1.07	0.41	1.40	0.04	0.94	0.50	0.95	0.61	0.92	0.43
Ugp2	0.93	0.56	0.77	0.18	0.83	0.15	1.49	0.01	0.85	0.18	0.81	0.11	0.53	0.01
Actb	0.98	0.72	1.07	0.41	1.09	0.16	1.41	0.03	1.10	0.53	1.11	0.27	0.94	0.42
B2m	1.10	0.23	1.10	0.44	1.00	0.91	1.34	0.04	1.02	0.89	1.07	0.37	0.62	0.00
Hprt1	1.11	0.07	1.38	0.03	1.25	0.03	1.74	0.00	1.45	0.00	1.18	0.12	0.66	0.03
Ldha	0.98	0.90	0.86	0.31	0.88	0.38	1.11	0.51	0.62	0.01	0.79	0.11	1.08	0.60
Rplp1	1.01	0.93	1.13	0.34	1.12	0.26	1.51	0.00	1.09	0.08	1.02	0.58	0.76	0.01

Appendix B: significant up/down regulation of 82 genes in cholesterol metabolism and lipoprotein signaling as a response to NCM treatment, body weight, and diet

GENE	LNV LNL		LNV LNH		LNL LNH		LHV LHL		LHV LHH		LHL LHH		LNV LHV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Abca1	1.06	0.79	1.62	0.05	1.33	0.16	0.95	0.54	1.08	0.79	1.06	0.67	1.27	0.74
Abca2	0.78	0.33	1.24	0.79	1.40	0.32	0.85	0.17	1.30	0.93	1.43	0.05	1.00	0.71
Abcg1	1.07	0.86	1.92	0.01	1.57	0.15	0.99	0.92	1.56	0.55	1.48	0.02	1.06	0.94
Acaa2	0.87	0.47	1.61	0.01	1.61	0.09	0.80	0.06	2.09	0.06	2.45	0.00	0.91	0.38
Akr1d1	1.15	0.44	1.68	0.00	1.27	0.23	0.91	0.47	1.30	0.40	1.34	0.33	1.15	0.77
Angptl3	1.31	0.35	1.93	0.00	1.29	0.13	1.22	0.44	1.67	0.01	1.29	0.66	0.97	0.86
Ankra2	0.90	0.57	1.66	0.00	1.61	0.05	1.00	0.96	1.61	0.11	1.51	0.25	0.87	0.52
Apoa1	0.35	0.02	0.39	0.00	0.97	0.67	0.38	0.00	0.44	0.06	1.08	0.99	0.68	0.24
Apoa2	1.00	0.75	1.39	0.05	1.21	0.30	1.10	0.85	1.50	0.03	1.28	0.92	0.75	0.53
Apoa4	0.16	0.08	0.47	0.28	2.64	0.53	0.72	0.67	0.78	0.36	1.02	0.72	0.60	0.29
Apob	0.78	0.54	1.16	0.28	1.31	0.14	0.94	0.84	1.33	0.14	1.32	0.87	0.74	0.46
Apoc1	0.88	0.96	1.18	0.47	1.17	0.41	1.12	0.84	1.44	0.01	1.20	0.75	0.65	0.41
Apoc3	0.87	0.39	1.33	0.45	1.33	0.22	0.86	0.64	1.08	1.00	1.18	0.91	0.91	0.45
Apod	0.75	0.75	0.82	0.64	0.95	0.92	0.82	0.40	0.88	0.46	1.00	0.56	0.95	0.55
ApoE	1.01	0.95	1.90	0.16	1.65	0.25	0.74	0.35	2.09	0.68	2.65	0.00	0.81	0.54
ApoF	0.94	0.83	1.13	0.02	1.05	0.82	0.91	0.41	1.07	0.76	1.10	0.79	1.12	0.87
Apol2	1.59	0.29	1.94	0.11	1.07	0.76	1.66	0.26	1.50	0.52	0.85	0.42	1.18	0.71
Cdh13	1.21	0.92	2.06	0.02	1.49	0.80	0.45	0.20	1.82	0.19	3.79	0.06	1.65	0.52
Cel	5.02	0.34	2.24	0.19	0.39	0.39	1.23	0.43	1.28	0.46	0.98	0.93	0.92	0.60
Cela3b	1.08	0.75	1.20	0.97	0.97	0.96	0.69	0.24	1.45	0.71	1.97	0.52	0.93	0.77
Cnbp	0.93	0.67	1.69	0.00	1.58	0.09	0.96	0.79	1.79	0.06	1.74	0.01	0.84	0.37
Colec12	0.75	0.56	1.08	0.52	1.26	0.22	0.89	0.37	1.49	0.20	1.57	0.10	0.75	0.30
Cxcl16	0.91	0.55	1.08	0.61	1.03	0.85	0.79	0.12	1.40	0.39	1.66	0.04	0.77	0.32
Cyb5r3	1.18	0.39	2.45	0.00	1.81	0.02	1.09	0.67	1.81	0.16	1.55	0.04	1.05	0.84
Cyp11a1	0.98	0.78	1.33	0.56	1.19	0.62	3.44	0.30	1.85	0.26	0.50	0.29	0.99	0.61
Cyp39a1	1.47	0.05	2.86	0.00	1.70	0.03	1.34	0.01	2.49	0.00	1.74	0.01	1.16	0.52
Cyp46a1	0.97	0.58	1.21	0.44	1.08	0.76	0.90	0.72	1.23	0.92	1.29	0.61	1.05	0.80
Cyp51	1.79	0.08	2.39	0.00	1.17	0.55	1.22	0.26	1.42	0.16	1.09	0.77	2.59	0.01
Cyp7a1	1.56	0.21	2.04	0.02	1.14	0.93	1.85	0.08	2.40	0.03	1.22	0.46	0.74	0.40
Cyp7b1	0.98	0.86	1.30	0.14	1.16	1.00	1.20	0.78	0.99	0.83	0.78	0.24	0.76	0.93
Dhcr24	1.98	0.01	3.55	0.00	1.56	0.17	1.16	0.65	2.19	0.28	1.77	0.03	2.51	0.01
Dhcr7	1.66	0.02	2.69	0.00	1.42	0.25	1.21	0.18	1.89	0.06	1.46	0.05	1.79	0.03
Ebp	1.35	0.28	2.50	0.00	1.61	0.01	1.12	0.51	1.81	0.06	1.51	0.20	1.14	0.97
Fdft1	1.56	0.04	2.48	0.00	1.38	0.09	1.43	0.11	2.47	0.00	1.61	0.00	1.44	0.12
Fdps	1.67	0.16	1.97	0.00	1.03	0.97	1.20	0.49	1.41	0.26	1.10	0.45	2.29	0.05
Hdlbp	0.85	0.24	1.44	0.41	1.48	0.25	0.89	0.48	1.47	0.93	1.55	0.02	0.98	0.76
Hmgcr	1.97	0.07	2.02	0.01	0.89	0.47	1.29	0.49	1.81	0.17	1.32	0.11	1.67	0.18
Hmgcs1	1.13	0.88	1.71	0.02	1.32	0.30	0.83	0.23	1.22	0.88	1.38	0.17	1.74	0.08
Hmgcs2	0.72	0.12	1.76	0.04	2.15	0.01	0.77	0.09	1.67	0.69	2.03	0.00	1.03	0.95
Idi1	2.09	0.24	1.68	0.12	0.70	0.21	1.23	0.82	1.01	0.92	0.77	0.31	3.22	0.07
Il4	0.86	0.83	0.71	0.22	0.72	0.15	0.87	0.79	0.71	0.63	0.77	0.40	0.72	0.75
Insig1	0.93	0.55	1.90	0.04	1.79	0.15	1.25	0.32	2.26	0.03	1.70	0.18	1.35	0.65
Insig2	0.63	0.24	1.63	0.03	2.27	0.17	0.58	0.30	1.52	0.51	2.46	0.26	0.85	0.53
Lcat	0.74	0.16	1.16	0.08	1.37	0.21	0.76	0.03	1.10	0.80	1.36	0.12	0.80	0.23
Ldlr	1.11	0.57	1.94	0.09	1.53	0.32	1.24	0.42	1.25	0.69	0.94	0.70	1.23	0.34
Ldlrap1	0.90	0.44	1.64	0.23	1.59	0.18	1.02	0.88	1.54	0.80	1.42	0.02	0.83	0.24
Lep	0.61	0.72	0.65	0.47	0.92	0.98	1.79	0.61	2.26	0.46	1.18	0.76	0.50	0.89
Lipe	0.93	0.80	1.66	0.03	1.55	0.16	0.93	0.52	1.87	0.58	1.88	0.01	1.10	0.81
Lrp10	1.01	0.98	1.96	0.24	1.69	0.23	0.81	0.38	1.63	0.88	1.88	0.00	1.11	0.86
Lrp12	1.07	0.77	1.83	0.01	1.50	0.14	0.68	0.01	1.39	0.66	1.93	0.00	1.19	0.61
Lrp1b	1.00	0.51	1.08	0.85	0.95	0.63	1.06	0.67	0.99	0.82	0.87	0.68	0.99	0.65
Lrp6	0.81	0.16	1.67	0.00	1.81	0.10	0.90	0.29	1.48	0.70	1.54	0.01	0.87	0.26
Lrpap1	0.81	0.58	1.22	0.13	1.31	0.09	0.90	0.67	1.35	0.22	1.40	0.49	0.88	0.61
Mbtps1	0.89	0.76	2.19	0.04	2.16	0.14	0.85	0.66	2.52	0.39	2.79	0.00	0.80	0.40
Mvd	1.08	0.55	1.30	0.44	1.06	0.91	1.02	0.94	1.12	0.99	1.03	0.90	1.61	0.16
Mvk	0.99	0.87	1.46	0.14	1.29	0.43	1.26	0.20	1.75	0.23	1.30	0.32	1.20	0.57
Nr0b2	1.23	0.46	3.44	0.00	2.44	0.11	2.04	0.04	3.18	0.13	1.46	0.20	0.67	0.16

GENE	LNV LNL		LNV LNH		LNL LNH		LHV LHL		LHV LHH		LHL LHH		LNV LHV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Nr1h2	0.86	0.38	1.36	0.05	1.38	0.20	0.99	0.95	1.56	0.39	1.47	0.16	0.83	0.31
Nr1h3	0.91	0.54	1.68	0.01	1.60	0.24	0.90	0.45	1.70	0.54	1.77	0.00	0.84	0.21
Nr1h4	0.93	0.61	1.43	0.04	1.35	0.46	0.90	0.74	1.42	0.55	1.47	0.46	0.75	0.35
Nsdhl	1.02	0.85	1.45	0.18	1.24	0.46	0.79	0.28	1.06	0.54	1.26	0.05	1.40	0.27
Olr1	0.63	0.15	0.53	0.01	0.73	0.21	0.27	0.06	0.64	0.14	2.21	0.06	0.84	0.94
Osbpl1a	1.02	0.96	1.70	0.00	1.46	0.12	0.84	0.12	1.44	0.39	1.60	0.04	1.03	0.99
Osbpl5	0.77	0.74	1.36	0.40	1.54	0.38	1.12	0.48	1.60	0.34	1.34	0.35	1.04	0.86
Pcsk9	2.43	0.02	3.38	0.02	1.21	0.99	1.67	0.27	1.51	0.65	0.85	0.64	4.07	0.05
Pmvk	1.09	0.69	2.91	0.00	2.34	0.07	1.01	0.98	2.08	0.26	1.92	0.00	1.34	0.07
Ppard	0.87	0.70	1.81	0.13	1.83	0.25	0.82	0.42	1.68	0.73	1.92	0.03	1.13	0.45
Prkaa1	1.09	0.78	2.17	0.00	1.74	0.08	1.09	0.65	2.41	0.01	2.07	0.01	0.90	0.63
Prkaa2	0.91	0.52	1.69	0.01	1.61	0.11	0.90	0.44	1.63	0.57	1.70	0.00	0.84	0.39
Prkag2	0.89	0.46	1.36	0.10	1.33	0.24	1.13	0.62	1.54	0.44	1.27	0.71	0.84	0.62
GD15649	1.00	0.51	1.08	0.86	0.95	0.63	1.00	0.56	0.99	0.82	0.93	0.78	0.99	0.65
Scap	0.77	0.29	1.24	0.17	1.41	0.23	1.09	0.71	1.50	0.51	1.29	0.63	0.70	0.25
Scarf1	0.89	0.38	1.77	0.00	1.74	0.04	0.76	0.07	1.35	0.82	1.67	0.04	1.22	0.63
Snx17	0.80	0.18	1.61	0.03	1.76	0.09	0.84	0.23	1.62	0.80	1.82	0.00	0.90	0.38
Soat1	0.97	0.76	1.49	0.00	1.34	0.69	0.65	0.03	1.25	0.85	1.80	0.01	0.96	0.62
Soat2	1.04	0.73	2.57	0.00	2.17	0.04	1.23	0.19	2.42	0.10	1.84	0.00	0.95	0.63
Sorl1	0.81	0.89	1.50	0.34	1.61	0.34	4.32	0.34	1.54	0.77	0.33	0.34	0.91	1.00
Srebf1	1.54	0.31	5.36	0.01	3.04	0.04	1.32	0.53	2.74	0.25	1.95	0.12	2.16	0.22
Srebf2	1.46	0.21	2.49	0.15	1.49	0.53	1.35	0.62	2.19	0.98	1.52	0.36	1.79	0.13
Stab2	0.95	0.97	1.07	0.47	0.99	0.58	1.42	0.37	2.40	0.35	1.59	0.33	0.70	0.44
Stard3	1.12	0.59	1.45	0.26	1.13	0.57	1.30	0.28	1.58	0.53	1.13	0.77	1.01	0.81
Tm7sf2	1.85	0.24	4.02	0.00	1.90	0.01	1.71	0.23	2.01	0.01	1.10	0.70	1.58	0.44
Trerf1	1.42	0.37	0.84	0.81	0.52	0.25	1.13	0.44	1.41	0.40	1.17	0.76	0.87	0.80
Vldlr	0.94	0.76	1.47	0.07	1.37	0.24	1.08	0.83	1.95	0.02	1.69	0.17	0.85	0.56
Actb	1.22	0.28	1.85	0.01	1.33	0.40	0.83	0.20	1.44	0.70	1.63	0.02	1.27	0.69
B2m	1.18	0.61	1.75	0.03	1.30	0.18	0.96	0.88	1.26	0.24	1.23	0.83	1.05	0.65
Hprt1	1.37	0.43	1.73	0.04	1.10	0.79	1.13	0.86	1.91	0.01	1.58	0.67	1.11	0.81
Ldha	0.50	0.00	0.86	0.13	1.51	0.15	0.54	0.00	0.88	0.38	1.51	0.08	0.76	0.16
Rplp1	0.92	0.98	1.40	0.09	1.33	0.10	0.90	0.78	1.46	0.14	1.51	0.34	0.94	0.67

GENE	ONV ONL		ONV ONH		ONL ONH		OHV OHL		OHV OHH		OHL OHH		LNV ONV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Abca1	0.88	0.53	0.43	0.01	0.50	0.01	1.14	0.59	0.35	0.06	0.25	0.04	1.49	0.13
Abca2	0.93	0.74	0.50	0.03	0.56	0.07	1.73	0.28	0.63	0.59	0.21	0.08	1.31	0.98
Abcg1	0.80	0.25	0.50	0.02	0.64	0.08	1.49	0.67	1.41	0.65	0.69	0.59	1.31	0.39
Acaa2	0.87	0.41	0.78	0.30	0.91	0.60	1.33	0.04	1.11	0.40	0.79	0.47	1.04	0.89
Akr1d1	0.92	0.73	0.79	0.25	0.87	0.40	1.01	0.90	0.75	0.87	0.55	0.79	1.26	0.24
Angptl3	0.93	0.80	0.67	0.08	0.74	0.13	2.04	0.14	0.96	0.79	0.56	0.36	1.70	0.01
Ankra2	0.90	0.63	0.75	0.15	0.85	0.40	1.37	0.23	0.38	0.66	0.26	0.52	1.07	0.54
Apoa1	0.42	0.10	0.28	0.09	0.67	0.77	0.74	0.45	0.39	0.01	0.50	0.08	0.69	0.03
Apoa2	1.07	0.97	1.02	0.96	0.97	0.96	1.34	0.62	1.08	0.92	0.68	0.55	0.61	0.10
Apoa4	0.78	0.28	0.18	0.12	0.23	0.50	0.80	0.44	0.43	0.56	0.34	0.32	1.36	0.31
Apob	0.84	0.46	0.69	0.06	0.85	0.31	2.32	0.21	0.85	0.51	0.62	0.49	0.72	0.08
Apoc1	0.88	0.69	0.80	0.43	0.92	0.89	1.38	0.57	1.24	0.93	0.86	0.84	0.80	0.21
Apoc3	0.63	0.25	0.21	0.01	0.34	0.02	1.42	0.71	0.04	0.05	0.06	0.00	1.02	0.64
Apod	1.58	0.48	1.14	0.98	0.73	0.41	1.28	0.46	1.17	0.68	0.60	0.79	1.15	0.74
Apoe	0.55	0.42	0.23	0.00	0.42	0.13	1.20	0.89	0.47	0.48	0.20	0.10	1.27	0.90
Apof	0.67	0.19	0.36	0.00	0.54	0.10	0.93	0.55	0.44	0.00	0.43	0.01	1.18	0.16
Apol2	0.79	0.98	1.05	0.54	1.37	0.60	0.94	0.73	0.59	0.39	0.47	0.57	1.64	0.17
Cdh13	2.00	0.14	1.26	0.29	0.65	0.31	3.32	0.07	2.00	0.11	0.48	0.28	4.34	0.00
Cel	0.92	0.70	0.48	0.37	0.53	0.38	1.05	0.52	1.12	0.75	0.66	0.80	1.22	0.45
Cela3b	0.96	0.91	1.07	0.68	1.14	0.51	1.71	0.39	1.29	0.44	1.01	0.93	1.24	0.65
Cnbp	1.01	0.71	0.90	0.63	0.91	0.88	1.22	0.72	1.09	0.47	0.73	0.28	0.91	0.72
Colec12	0.91	0.61	0.75	0.18	0.85	0.28	0.76	0.61	0.94	0.55	1.29	0.35	0.93	0.79
Cxcl16	0.68	0.16	0.69	0.34	1.04	0.76	1.62	0.33	1.08	0.54	0.96	0.97	1.19	0.36
Cyb5r3	0.75	0.12	0.77	0.14	1.05	0.68	1.23	0.45	0.66	0.16	0.48	0.00	1.73	0.01

GENE	ONV ONL		ONV ONH		ONL ONH		OHV OHL		OHV OHH		OHL OHH		LNV ONV	
	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value	Fold change	p- value
Cyp11a1	0.82	0.50	0.78	0.96	0.97	0.58	1.22	0.82	0.76	0.70	0.51	0.86	1.86	0.19
Cyp39a1	0.91	0.60	0.46	0.02	0.52	0.01	1.11	0.97	0.15	0.14	0.08	0.03	2.11	0.00
Cyp46a1	0.58	0.33	0.99	0.45	1.76	0.26	0.71	0.75	0.90	0.97	1.19	0.94	1.40	0.29
Cyp51	0.77	0.44	0.63	0.09	0.83	0.44	2.22	0.11	0.96	0.77	0.46	0.12	1.42	0.10
Cyp7a1	0.72	0.29	0.33	0.25	0.47	0.79	0.86	0.88	0.27	0.68	0.13	0.25	1.22	0.41
Cyp7b1	0.69	0.17	0.64	0.17	0.94	0.93	1.49	0.29	0.84	0.45	0.62	0.47	0.57	0.03
Dhcr24	0.84	0.53	0.58	0.07	0.71	0.18	1.45	0.38	0.29	0.65	0.14	0.39	3.87	0.00
Dhcr7	0.95	0.66	0.72	0.35	0.78	0.57	1.45	0.36	1.09	0.47	0.73	0.63	3.01	0.01
Ebp	0.75	0.33	0.71	0.09	0.97	0.91	2.23	0.18	0.52	0.24	0.28	0.00	1.05	0.69
Fdft1	0.72	0.41	0.76	0.92	1.08	0.41	1.57	0.37	0.86	0.43	0.41	0.33	1.26	0.34
Fdps	0.59	0.23	0.73	0.24	1.26	0.40	1.74	0.25	0.81	0.79	0.41	0.00	2.03	0.08
Hdlbp	0.69	0.10	0.61	0.05	0.90	0.87	1.14	0.65	0.70	0.70	0.51	0.03	1.61	0.19
Hmger	0.61	0.08	0.37	0.04	0.62	0.15	1.49	0.24	0.49	0.39	0.24	0.01	3.92	0.05
Hmgcs1	1.00	0.96	0.65	0.31	0.67	0.21	1.46	0.05	0.79	0.91	0.40	0.10	1.30	0.20
Hmgcs2	0.80	0.23	0.49	0.09	0.63	0.17	0.95	0.61	0.88	0.88	0.62	0.14	1.32	0.35
Idi1	0.94	0.77	0.92	0.53	1.00	0.58	1.91	0.46	1.20	0.88	0.69	0.67	1.05	0.99
Il4	0.92	0.61	0.87	0.74	0.96	0.84	0.67	0.45	0.51	0.36	0.86	0.97	0.27	0.02
Insig1	0.75	0.12	0.85	0.73	1.17	0.43	0.55	0.36	0.52	0.33	1.09	0.45	2.22	0.10
Insig2	0.89	0.40	0.52	0.13	0.60	0.13	0.90	0.77	0.51	0.30	0.54	0.57	0.63	0.14
Lcat	0.68	0.20	0.40	0.01	0.59	0.15	0.93	0.88	0.54	0.04	0.51	0.00	0.99	0.86
Ldlr	0.58	0.24	0.44	0.08	0.78	0.67	1.47	0.31	0.54	0.74	0.34	0.07	2.06	0.10
Ldlrap1	0.78	0.26	0.66	0.06	0.87	0.56	1.23	0.79	0.83	0.94	0.54	0.12	1.75	0.13
Lep	1.34	0.57	0.50	0.45	0.38	0.29	1.16	0.96	0.79	0.29	0.71	0.54	0.91	0.97
Lipe	0.96	0.64	0.89	0.73	0.95	0.86	1.72	0.31	1.19	0.79	0.57	0.00	1.42	0.12
Lrp10	0.62	0.52	0.27	0.01	0.45	0.12	1.10	0.85	0.15	0.22	0.08	0.09	1.41	0.76
Lrp12	0.89	0.52	0.40	0.06	0.46	0.01	1.28	0.95	0.14	0.20	0.08	0.10	1.29	0.38
Lrp1b	1.24	0.73	1.05	0.52	0.86	0.75	0.85	0.55	0.97	0.74	1.10	0.90	0.99	0.95
Lrp6	0.81	0.24	0.43	0.01	0.55	0.03	1.07	0.91	0.62	0.14	0.51	0.06	1.00	1.00
Lrpap1	0.77	0.33	0.60	0.05	0.79	0.26	1.36	0.19	0.45	0.92	0.21	0.03	0.75	0.08
Mbtps1	0.63	0.12	0.42	0.03	0.69	0.42	1.44	0.64	0.97	0.53	0.48	0.75	1.43	0.53
Mvd	0.69	0.02	0.77	0.12	1.14	0.48	2.04	0.15	1.27	0.41	0.54	0.13	1.92	0.06
Mvk	0.71	0.18	0.49	0.16	0.71	0.90	0.83	0.38	0.78	0.93	0.59	0.52	2.18	0.02
Nr0b2	1.27	0.47	0.84	0.95	0.68	0.62	1.08	0.76	0.68	0.70	0.44	0.01	0.77	0.62
Nr1h2	0.84	0.41	0.74	0.12	0.90	0.60	1.38	0.40	0.96	0.68	0.74	0.23	1.23	0.20
Nr1h3	0.73	0.17	0.59	0.05	0.83	0.55	1.25	0.61	0.76	0.93	0.49	0.08	1.38	0.09
Nr1h4	0.59	0.10	0.49	0.04	0.85	0.45	5.04	0.07	0.53	0.31	0.22	0.00	1.00	0.80
Nsdhl	0.62	0.08	0.21	0.00	0.35	0.01	0.33	0.63	0.20	0.19	0.54	0.15	2.01	0.04
Olr1	0.70	0.18	0.24	0.40	0.35	0.64	0.77	0.78	0.32	0.07	0.35	0.42	1.94	0.20
Osbpl1a	0.72	0.16	0.33	0.01	0.48	0.02	0.77	0.24	0.67	0.13	0.61	0.02	2.15	0.00
Osbpl5	1.06	0.91	0.71	0.38	0.68	0.39	1.89	0.06	1.27	0.44	0.57	0.52	1.45	0.18
Pcsk9	1.03	0.76	1.00	0.88	1.00	0.89	1.43	0.38	0.97	0.54	0.59	0.93	5.90	0.06
Pmvk	0.67	0.23	0.56	0.22	0.85	0.92	1.43	0.32	0.69	0.61	0.42	0.31	1.98	0.02
Ppard	0.79	0.40	0.46	0.12	0.60	0.43	1.24	0.30	0.56	0.52	0.37	0.04	1.63	0.07
Prkaa1	0.81	0.26	0.80	0.29	1.02	0.93	1.45	0.41	1.57	0.05	0.96	0.97	1.56	0.00
Prkaa2	0.66	0.09	0.42	0.01	0.65	0.38	1.53	0.31	0.73	0.85	0.44	0.03	1.28	0.12
Prkag2	0.71	0.15	0.79	0.23	1.13	0.39	1.56	0.24	0.88	0.72	0.52	0.02	1.35	0.07
GD15649	1.00	0.81	0.98	0.54	1.00	0.69	0.97	0.52	0.98	0.72	1.08	0.90	1.00	1.00
Scap	0.72	0.26	0.62	0.06	0.89	0.76	2.80	0.14	0.90	0.81	0.49	0.00	0.64	0.01
Scarf1	0.62	0.04	0.33	0.02	0.55	0.10	1.31	0.82	0.45	0.18	0.25	0.09	1.65	0.01
Snx17	0.71	0.16	0.28	0.00	0.40	0.02	0.88	0.50	0.18	0.08	0.13	0.10	1.13	0.64
Soat1	0.77	0.17	0.29	0.01	0.39	0.02	0.95	0.62	1.12	0.52	0.90	0.77	1.49	0.03
Soat2	0.76	0.24	0.49	0.01	0.66	0.22	1.40	0.31	0.90	0.93	0.58	0.20	2.02	0.00
Sorl1	0.60	0.91	0.31	0.63	0.52	0.54	0.26	0.26	0.61	0.32	1.82	0.88	5.27	0.03
Srebf1	0.59	0.43	0.30	0.04	0.53	0.25	0.80	0.31	0.74	0.74	0.49	0.92	10.82	0.02
Srebf2	0.92	0.93	0.75	0.98	0.83	0.94	1.05	0.99	0.62	0.80	0.44	0.05	2.09	0.31
Stab2	0.63	0.20	0.64	0.45	1.04	0.36	1.43	0.33	1.64	0.27	0.95	0.74	1.73	0.10
Stard3	0.91	0.44	0.74	0.11	0.84	0.31	1.38	0.39	0.65	0.25	0.44	0.23	0.99	0.70
Tm7sf2	0.88	0.81	0.98	0.70	1.14	0.53	4.49	0.15	0.40	0.50	0.12	0.12	1.32	0.21
Trerf1	0.79	0.48	0.89	0.67	1.16	0.45	1.75	0.42	0.84	0.43	0.54	0.29	0.99	0.81
Vldlr	0.97	0.83	1.03	0.61	1.08	0.49	1.31	0.45	1.53	0.45	1.35	0.73	1.72	0.04
Actb	0.74	0.08	0.35	0.01	0.48	0.02	1.65	0.55	0.17	0.17	0.09	0.06	1.94	0.01
B2m	0.80	0.38	0.48	0.19	0.61	0.36	1.12	0.82	0.16	0.39	0.10	0.66	0.97	0.67
Hprt1	0.86	0.63	0.67	0.92	0.79	0.68	1.25	0.63	1.93	0.57	1.32	0.92	0.92	0.67
Ldha	0.62	0.11	0.38	0.03	0.62	0.14	0.97	0.71	0.56	0.18	0.50	0.07	1.15	0.13
Rplp1	0.75	0.31	0.64	0.11	0.87	0.29	1.36	0.36	1.18	0.53	0.72	0.39	0.80	0.19